American Journal of

Plant Physiology

ISSN 1557-4539

Academic
Journals Inc.

www.academicjournals.com

American Journal of Plant Physiology 4 (1): 18-23, 2009
ISSN 1557-4539

Study on the Diurnal Changes of Net Photosynthetic Rate and the Impact Factors of *Stevia rebaudiana* Bertoni in Autumn

Lv Chengguo, Ma Lei and Shi Yan
Dryland Technology Key Laboratory, Qingdao Agricultural University,
Shandong Province, Qingdao, 266109, China

Abstract: Relationship between diurnal changes of net photosynthetic rate (Pn) and the impact factors in leaves of *Stevia rebaudiana* Bertoni were studied. Diurnal changes of Pn and environmental factors (photosynthetic available radiation and temperature and relative humidity) and physiology factors (stomatal conductance and transpiration rate and intercellular CO_2) in leaves of *Stevia rebaudiana* Bertoni chicory were measured using LI-6400 portable photosynthesis system. The relationship between Pn and environmental factors were analyzed by regression analysis and path analysis. The results showed that the curve of diurnal changes of Pn was demonstrated two peaks in clear day and appeared midday depression at noon and the stomatal conductance decreased. All those were due to high photorespiration which was caused by high light intensity and high temperature.

Key words: *Stevia rebaudiana* Bertoni, photosynthetic rate, diurnal changes, impact factors, midday depression

INTRODUCTION

Recently, an increasing demand has been noted for new natural substitute sweeteners for sucrose and synthetic sweeteners such as saccharine and aspartame. Stevioside is a sweetener of plant origin possessing a 200-350 times higher sweetening property than sucrose. Stevioside comes from the South American perennial shrub *Stevia rebaudiana* Bertoni and it is the most abundant among the nine known sweet glycosides of the plant (Crammer and Ikan, 1986). As reviewed by Geuns (2003), stevioside is safe when used as a sweetener and there are no side effects like mutagenicity, carcinogenicity or teratogenicity.

Leaves of *Stevia rebaudiana* Bertoni have been used for the last 20 years in countries of South America and Southeast Asia as a low-calorie sugar substitute (Young, 2002). Their sweetness is due to the glycosides stevioside, steviolbioside and rebaudiosides A, B, C, D, E, F and dulcoside A (Brandle *et al*., 2002). Stevioside is 300 times sweeter than sugar but has a bitter aftertaste (Brandle *et al*., 1992; Podporinova, 2004). The sweetness of rebaudiosides increases with increasing amount of sugar units bonded to the aglycon (steviol). However, their content in the plant material decreases at the same time (Brandle and Rosa, 1992; Brandle *et al*., 1992; Tolstikov and Kovylyaeva, 2007).

However, the photosynthetic rate and the impact factors of *Stevia rebaudiana* grown in China in autumn rarely been studied. Therefore, the goal of the present study was to determine the diurnal changes of net photosynthetic rate and the impact factors of *Stevia rebaudiana* of Chinese region.

Corresponding Author: Shi Yan, Dryland Technology Key Laboratory, Qingdao Agricultural University, Shandong Province, Qingdao, 266109, China

MATERIALS AND METHODS

Materials

The research was conducted in greenhouse with thick soil layer, Plant Science and Technology College, Qingdao Agricultural University (36°15'N, 120°20'E), where the climate is temperate. The annual average temperature is 16°C, August was the highest temperature month during the period, which was average 25.1°C and the lowest temperature was in January, which was averaged -1.2°C. The mean annual precipitation is 775.6 mm and most of which falling between July to September. The organic matter containing of the soil is 1.24%, total nitrogen is 1.04%, available nitrogen, available phosphorus and available potassium are 86.54, 24.58 and 85.72 mg kg^{-1}, respectively.

No. 1 Qingtian (*Stevia rebaudiana* Bertoni) was used in all experimentation.

Methods

The photosynthetic characters of leaves in *Stevia rebaudiana* were studied with LI-6400 portable photosynthesis system (made by LI-COR Biosciences, USA) in middle September, 2007. Five plants were repetitive measured and 2 healthy functional leaves at the same leaf position were randomly selected every time and measured 3 times every leaf. The result of each test was average to get the mean value.

The measurement index were: net photosynthetic rate/(Pn, µmol CO_2/m^2/sec), transpiration rate/(Tr, mmol H_2O/m^2/sec), leaf to air vapour pressure deficient/(Vpd, kPa), leaf temperature/ (Tleaf,°C), photosynthetically active radiation/(PAR, µmol mol^{-1}), Intercellular CO_2 contain/ (Ci, µmol mol^{-1}), air CO_2 contain/(Ca, µmol mol^{-1}), stomatal conductance/(Cs, mmol/m^2/sec), air relative humidity/(RH, %).

RESULTS

Diurnal Changes of Net Photosynthetic Rate and Transpiration Rate of *Stevia rebaudiana* in Greenhouse in Autumn

Diurnal variation of photosynthetic rate (Pn) (Fig. 1) presented a bimodal curve and obvious midday depression phenomenon occurred. The first peak value was the highest, which occurred at about 12:00 am, the net photosynthetic rate was 13.4 µmol CO_2/m^2/sec, thereafter Pn reduced quickly and to the minimum value was 10.8 µmol CO_2/m^2/sec and the second peak value occurred at 14:00 pm, the net photosynthetic rate was 13.4 µmol CO_2/m^2/sec and decreased after then. The first peak was

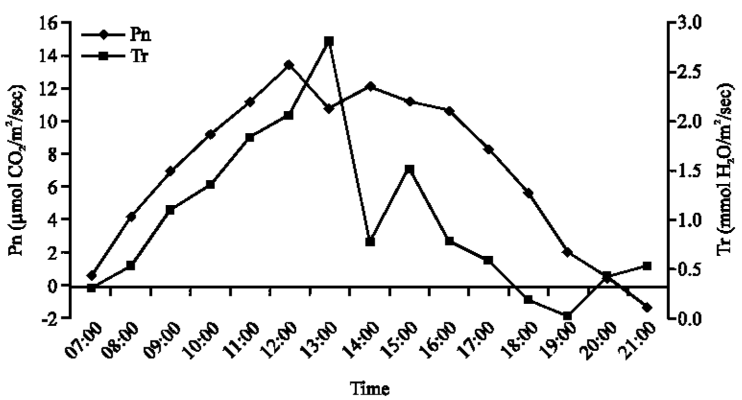

Fig. 1: Diurnal changes of photosynthetic rate in leaves of *Stevia rebaudiana*

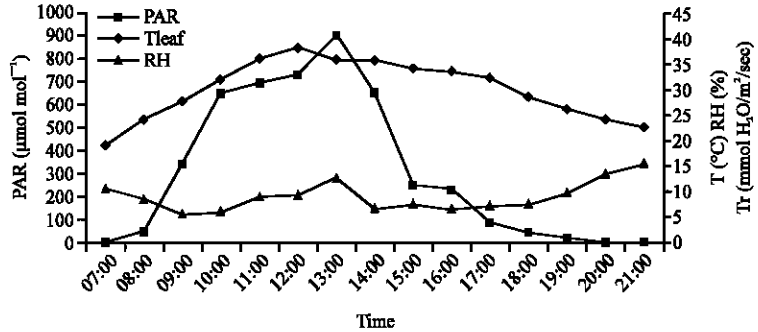

Fig. 2: Diurnal changes of photosynthetic available radiation and air temperature and transpiration rate and relative humidity

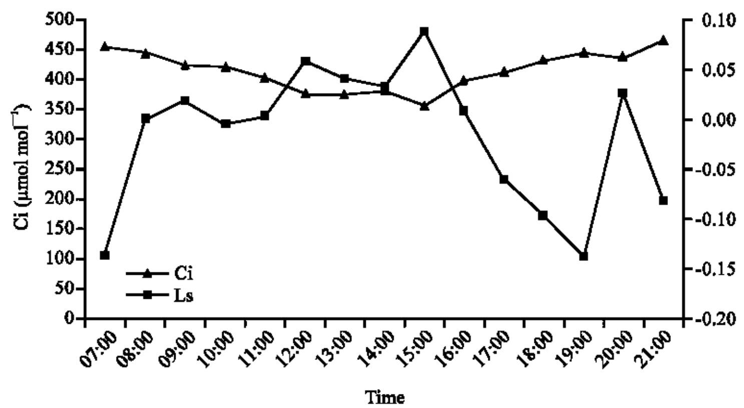

Fig. 3: Diurnal changes of intercellular CO_2 and stomatal constraint in leaves of *Stevia rebaudiana*

90% higher than the second peak by comparing the results, there was obvious midday depression of photosynthesis. Pn became negative after 8:00 pm. The transpiration rate followed closely the Pn course during the day. It presented a bimodal curve, too.

Diurnal Changes of the Main Environmental Factors

Plants photosynthesis appears complex concentration profiles affected by many factors. Figure 2 shows that the change of PAR is a single-peak curve, the highest PAR is at 1:00 pm in a day and then decreased gradually after-words in autumn; Leaf temperature increased first and the highest (36.91°C) appeared at about 1:00 pm, then dropped; The relative humidity was higher at 7:00 am, 2:00 and 8:00 pm than other time. The Vpd followed closely the PAR course during the day; however its peak appeared at 2:00 pm.

Stomatal movement is important for plants to exchange gas and water with environment. It has very close relation with photosynthesis, respiration and transpiration. According to the opinion of Sharkey and Ogawa (1987). If Gs and Ci decreased as Pn decreased and cause a decrease in stomatal limitation value (Ls), the stomatal limitation is dominant; If Ci increase as Gs and Ls decrease, nonstomatal limitation is the dominant one. Figure 3 and 4 show that Ci and Ls decreased and Gs increased as Pn decreased. The results showed that diurnal change in net photosynthetic rate (Pn) of *Stevia rebaudiana* leaves was a typical bimodal curve determinately regulated by stomatal conductance.

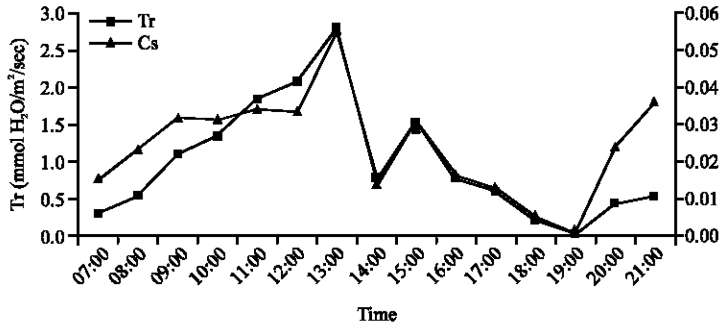

Fig. 4: Diurnal changes of stomatal conductance and transpiration rate in leaves of *Stevia rebaudiana*

Table 1: Path analysis among photosynthetic rate and main factors

Acting factor	Path coefficient								
	Direct effect	Gs	Ci	Tleaf	Ca	RH	PAR	Vpd	Tr
Gs	0.28008		0.10304	-0.08086	0.02704	-0.04848	-0.01760	0.19118	-0.16569
Cs	-0.27963	-0.10320		0.23154	0.07804	-0.04577	0.02003	-0.94910	0.14247
Tleaf	-0.25848	0.08762	0.25048		-0.07749	0.05625	-0.02290	1.06057	-0.14031
Ca	0.14438	0.05246	-0.15114	0.13873		-0.03437	0.00873	-0.71784	0.04734
RH	-0.13929	0.09748	-0.09189	0.10438	0.03563		0.00548	-0.57386	-0.00433
PAR	-0.02844	0.17332	0.19700	-0.20811	-0.04434	0.02686		0.84893	-0.17164
Vpd	1.09301	0.04899	0.24281	-0.25081	-0.09482	0.07313	-0.02209		-0.12653
Tr	-0.19630	0.23641	0.20295	-0.18476	-0.03482	-0.00307	-0.02486	0.70452	

Diurnal Changes of Stomatal Conductance and Transpiration Rate in Leaves of *Stevia rebaudiana*

Figure 4 show that diurnal variation of stomatal conductance (Gs) was a double peak curve, the peak (55.8 mmol/m^2/sec) of Gs appeared at 1:00 pm and the second crest value existed at about 3:00 pm, it was only 31.1 mmol/m^2/sec. Its pattern was the same with Pn.

Analysis of the Quantitative Relationship among Pn and Main Environmental Factors

The effect of environment factors on Pn was not isolated or individual but a result of integrate all factors affected. To make the further analysis of the quantitative relationship between Pn and main environmental factors, result was further investigated by making use of the regression analysis. The regression equation between Pn and its influence factors was:

$$Pn = 3.6885+95.14803Gs-0.04062Ci-0.21166T+0.03332Cs-0.2308RH-0.00042PAR+3.532Vpd-1.1953Tr \quad R = 0.9927^* \ (R_{0.05} = 0.9853)$$

The results approve that the regression equation is significant and the correlation is close, so it can be used in the practical operation. Regression analysis showed that there was a significant regression relationship between the Pn and its influence factors.

Table 1 shows that factors have ordinal influence on the diurnal change of Pn in Stevia rebaudiana: Vpd, Gs, Cs, PAR, RH, Tr, T and Ci. The Tleaf, Tr, PAR and Gs had indirect positive effect on the Pn via affecting Vpd. While Ci, Ca, RH had indirect negative effect on the Pn via affecting Vpd. So the Vpd is the most effective factor to Pn and the other factors affect the Pn via the Vpd.

DISCUSSION

The results showed that under natural conditions (control), diurnal course of net photosynthetic rate (Pn) in *Stevia rebaudiana* leaves presented two peaks and daily variation of stomatal conductance

(Gs) and transpiration rate (Tr) appeared two peaks; Pn increased with enhanced photosynthetically active radiation and stabilization when photosynthetically active radiation reaches certain level from sunrise. At 12:00 am, Pn appeared the peak when the environmental factors formed the most proper combination. However, PAR and temperature increased gradually when much higher light density and the high temperature inhibited Pn at 1:00 pm. It was not highest leaf temperature when the PAR was maximum because Tr and RH increased with PAR and they cause leaf temperature not to be too high. So midday depression of photosynthesis was not closely related with temperature in greenhouse. The main cause of midday depression of photosynthesis was due to higher light intensity which caused photorespiration increased and Pn decreased (Heber *et al*., 1987).

A physiological process characteristic of crop photosynthesis is a complex process. Pn is closely related with chlorophyll content, leaf thickness and leaf maturity and light intensity, temperature, relative humidity and moisture content in the soil play important roles, too. Pn in leaves of *Stevia rebaudiana* leaves varied in day time, it was double peak curve and there was obvious midday depression of photosynthesis. Midday depression is the character of C_3 plants. The lowest valley between 2 peaks is the result of high photorespiration under high light intensity and high temperature. It is also the reason that protective stomatal conductance and intercellular CO_2 concentration decrease. The function of stomata in plants is controlling gas exchange and modulating water balance, istomatal aperture and stomatal resistance play important roles in water status and CO_2 assimilation. Generally speaking, the Ci and Pn decrease as stomatal aperture decreases or the stomatal resistance increase and reducing stomatal opening status led transpiration rate decrease and water loss reduction; Only a few was reported non-stomatal regulation sometimes (Morison, 1987; Schulze *et al*., 1987; Bahrun *et al*., 2002).

The results show that Vpd, whose coefficient of determination is the highest, is a main factor affect to Pn. midday depression of photosynthesis was not closely related with temperature in greenhouse. The main cause of midday depression of photosynthesis was due to higher light intensity which inhibited photosynthesis and promoted photorespiration while the correlativity of temperature and midday depression of photosynthesis was small. It is in accordance with Raschke' theory (Pandey *et al*., 2003) that if Vpd was maintain constant the change of temperature at a certain range does not led to the obvious change of photosynthesis. In fact, the effect of temperature on photosynthesis is mediated via Vpd. Bisides, temperature can cause stomatal conductance and transpiration rate which has direct or indirect effect on photosynthesis (Raschke and Rseman, 1988).

REFERENCES

Bahrun, A., 2002. Drought-induced changes in xylem pH, ionic composition and ABA concentration act as early signals in field-grown maize (*Zea mays* L.). J. Experim. Bot., 53: 251-263.

Brandle, J.E and N. Rosa, 1992. Heritability for yield, leaf-stem ratio and stevioside content estimated from a landrace cultivar of *Stevia rebaudiana*. Can. J. Plant Sci., 72: 1263-1266.

Brandle, J.E., A.N. Starratt and M. Gijzen, 2002. Rebaudioside F, a diterpene glycoside from *Stevia rebaudiana*. Phytochemistry, 78: 527-531.

Crammer, B. and R. Ikan, 1986. Sweet glycosides from stevia plant. Chem. Br., 22: 915-916.

Geuns, J.M.C., 2003. Stevioside. Phytochemistry, 64: 913-921.

Heber, U., S. Neimanis and O.L. Lange, 1987. Stomatal aperture, photosynthesis and water fluxes in mesophyll cells as affected by the abscission of leaves. Simultaneous measurements of gas exchange, light scattering and chlorophyll fluorescence. Planta, 167: 554-562.

Morison, J.I.L., 1987. Intercellular CO_2 Concentration and Stomatal Response to ACO_2. In: Stomatal Function, Zieger, E., G.D. Farquhar and R. Cowan (Eds.). Standford University Press, Stand (CA), USA., ISBN: 0804713472, pp: 251-299.

Pandey, S., S. Kumar and P.K. Nagar, 2003. Photosynthetic Performance of *Ginkgo biloba* L. grown under high and low irradiance. Photosynthetica, 41: 505-511.

Raschke, K. and A. Rsemann, 1988. The midday depression of CO_2 assimilation in leaves of *Arbutos unedo* L. diurnal changes in photosynthetic capacity related to changes in temperature and humidity. Planta, 168: 546-558.

Schulze, E.D., N.C. Turnere, T. Gollan and K.A. Shackel, 1987. Stomatal responses to air humidity and soil drought. In: Stomatal Function, Zieger, E., G.D. Farquhar and I.R. Cowan (Eds.). Standford University Press, Stand (CA), USA., ISBN: 0804713472, pp: 311-321.

Sharkey, T.D. and T. Ogawa, 1987. Stomatal Responses to Light. In: Stomatal Function, Zieger, E., G.D. Farquhar and I.R. Cowan (Eds.). Standford University Press, Stand (CA), USA., ISBN: 0804713472, pp: 195-227.

Tolstikov, A.G. and G.I. Kovylyaeva, 2007. Glycosides from *Stevia rebaudiana*: Glycosides from *Stevia rebaudiana*. Chem. Natural Compounds, 433: 81-85.

Young, H.C., 2002. Supercritical fluid extraction and liquid chromatographic-electrospray mass spectrometric analysis of stevioside from *Stevia rebaudiana* leaves. Chromatographia, 55: 617-620.