BRIEF COMMUNICATION

The photosynthetic parameters of cucumber as affected by irradiances with different red:far-red ratios

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Abstract

We compared photosynthetic performance between cucumber (*Cucumis sativus* L.) leaves acclimated to saturating irradiances with high red : far red (R:FR = 10) and normal R:FR (= 1.4) ratios. The net photosynthetic rate (P_N) and stomatal conductance (g_s) of the leaves acclimated to high R:FR were greater than those of the leaves acclimated to normal R:FR; the greater g_s partly explains the greater P_N. The greater g_s of the high-R:FR-leaves probably resulted from a higher stomatal density and/or a greater size. P_N of the high R:FR leaves was still greater than that of the normal R:FR leaves at the same intercellular CO_2 concentration (c_i). This indicates that non-stomatal factors also increased the photosynthetic capacity of the high R:FR leaves. The maximum Rubisco carboxylase activity estimated from a P_N-c_i curve analysis was also greater in the high R:FR leaves, however, the intrinsic water-use efficiency ($WUE_i = P_N/g_s$) of the high R:FR leaves was lower than that of the normal R:FR leaves.

Additional key words: *Cucumis sativus*, intrinsic water-use efficiency, Rubisco, stomatal conductance, stomatal density.

We previously reported that *Cucumis sativus* leaves acclimated to the radiation with a high red (660 ± 10 nm) to far-red (730 ± 10 nm) ratio (R:FR) show a higher net photosynthetic rate (P_N) at saturating irradiance than those acclimated to the radiation with the spectrum similar to sun radiation (Shibuya *et al*. 2010, 2012). In these reports, we concluded that the greater P_N mainly results from a greater biomass per unit leaf area. However, radiation with a higher R:FR has been reported to enhance stomatal density (Schoch *et al*. 1984, Boccalandro *et al*. 2009, Casson *et al*. 2009); thus, it is also possible that an increased stomatal conductance (g_s) contributed to the greater P_N *via* an increase in the intercellular CO_2 concentration (c_i). Therefore, the aim of this study was to distinguish stomatal and non-stomatal contributions to the increased P_N. In addition, we focused on water-use efficiency in these plants.

Cucumis sativus L. cv. Hokushin seedlings were acclimated from germination to radiation with high R:FR (= 10) or normal R:FR (= 1.4) in a growth chamber. The spectral composition of radiation was adjusted using LED panels containing a mixture of blue, green, red, and far-red LED elements (*CCS Inc*., Kyoto, Japan). The spectrum of each radiation (Fig. 1) was measured using a *BLK-CXR-SR* spectrometer (*StellarNet*, Tampa, FL, USA). The proportion of active phytochrome to total phytochrome, which was calculated from the spectral photon distribution and phytochrome photochemical cross-sections (Sager *et al*. 1988), was 0.846 in the high R:FR and 0.726 in the normal R:FR. The photosynthetic photon flux density (PPFD) was maintained at 300 μmol(photon) m^{-2} s^{-1} at the leaf surface. The photoperiod was 16-h. The air temperature and the relative humidity were maintained at 28 °C and 50 %, respectively. When the first true leaves had expanded and the second leaves had begun to emerge (13 d and 12 d after seeding under the high and normal R:FR, respectively), we measured P_N and g_s of the first true leaves with a photosynthesis system *LI-6400*

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Abbreviations: c_i - intercellular CO_2 concentration; g_s - stomatal conductance; LMA - dry leaf mass per unit area; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density; R:FR - red:far-red ratio; V_{cmax} - maximum Rubisco carboxylase activity; WUE_i - intrinsic water-use efficiency.

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(*LI-COR*, Lincoln, NE, USA) at ambient CO_2 concentrations of 75, 100, 200, 300, or 400 µmol mol^{-1} and PPFD of 2000 µmol(photon) m^{-2} s^{-1} provided by red and blue LEDs at a ratio of 9:1. We calculated c_i according to the method of von Caemmerer and Farquhar (1981). The maximum Rubisco carboxylase activity (V_{cmax}) was estimated from P_N-c_i curves using a curve fitting model developed by Sharkey *et al*. (2007). The intrinsic water-use efficiency (WUE_i) was calculated as P_N/g_s. The area and dry mass of the first true leaves from five plants in each treatment group were measured and used to calculate the dry leaf mass per area (LMA). We observed the adaxial and abaxial surfaces of five leaves using a digital microscope *VHX-1000* (*Keyence Corporation*, Osaka, Japan) at a 500× magnification and counted the numbers of stomata and epidermal cells in the field of view of two randomly selected parts of each leaf, then calculated the average for these fields of view. The stomata length was measured using an image processing software (*VHX-H1M1*; *Keyence Corp*.). The stomatal density was calculated by dividing the number of stomata by the area. We defined the stomatal index as the ratio of the number of stomata in a given area divided by the total number of stomata plus other epidermal cells (Casson *et al*. 2009). Significances of differences between treatment were tested using the Student's *t*-test.

P_N and g_s of the high R:FR leaves were 1.19 and 1.51 times higher, respectively, than those of normal R:FR leaves (Table 1). The greater g_s of the high R:FR leaves contributed to their greater P_N *via* an increase in c_i (Table 1). The adaxial stomatal density of the high R:FR leaves was 1.30 times higher than that of normal R:FR leaves (Table 2). The stomata lengths of the high R:FR leaves were 1.13 and 1.18 times higher than those

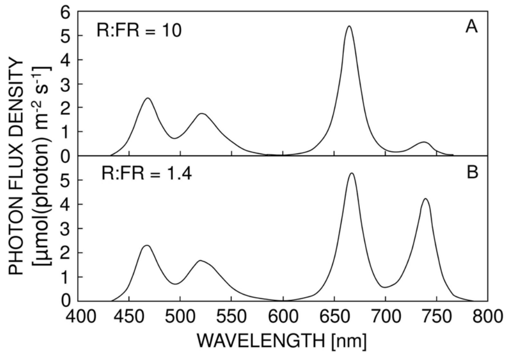

Fig. 1. Spectra of a LED panel with a high red:far-red (R:FR) ratio (= 10; *A*) or a normal R:FR ratio (= 1.4; *B*). R:FR was estimated by dividing the cumulative photon flux of R radiation (660 ± 10 nm) by that of FR radiation (730 ± 10 nm).

Table 1. Net photosynthetic rate (P_N) [µmol(CO_2) m^{-2} s^{-1}], stomatal conductance (g_s) [mmol(H_2O) m^{-2} s^{-1}], intercellular CO_2 concentration (c_i) [µmol mol^{-1}], maximum Rubisco carboxylase activity (V_{cmax}) [µmol m^{-2} s^{-1}], intrinsic water-use efficiency (WUE_i, = P_N/g_s) [µmol(CO_2) mol^{-1}(H_2O)], and leaf mass per unit area (LMA) [g(d.m.) m^{-2}] in *Cucumis sativus* first-true-leaves acclimated to irradiances with a high red:far-red (R:FR) ratio (= 10) or a normal R:FR ratio (= 1.4). Photosynthesis was measured at PPFD of 2 000 µmol(photon) m^{-2} s^{-1}, an ambient CO_2 concentration of 400 µmol mol^{-1}, a leaf temperature of 28 °C, and a 50 % relative humidity of air. Data are means ± SE, *n* = 10 (photosynthetic parameters) or 5 (LMA), ** - significant differences between the treatments at $P \leq 0.01$ according to the Student's *t*-test. The value determined by dividing the average P_N by the average g_s is not identical to the WUE_i value because of different averaging procedures.

Treatment	P_N	g_s	c_i	V_{cmax}	WUE_i	LMA
R:FR = 10	28.1 ± 0.7	587 ± 59	296 ± 4.3	150 ± 6	50.1 ± 2.8	27.6 ± 0.2
R:FR = 1.4	23.6 ± 0.4**	389 ± 22**	276 ± 4.3**	124 ± 9**	62.1 ± 2.7**	22.5 ± 1.2**

Table 2. Stomatal density [stomata mm^{-2}], stomatal index [relative], ratio of abaxial to adaxial stomata [relative], and stomata length [µm] in *Cucumis sativus* first-true-leaves acclimated to irradiances with a high red:far-red (R:FR) ratio (= 10) or a normal R:FR ratio (= 1.4). Data are means ± SE, *n* = 5, * and ** - significant differences between the treatments at $P \leq 0.05$ and $P \leq 0.01$, respectively, according to the Student's *t*-test.

Treatment	Stomatal density		Stomatal index		Abaxial/adaxial	Stomata length	
	adaxial	abaxial	adaxial	abaxial	ratio	adaxial	abaxial
R:FR = 10	634 ± 49	714 ± 62	0.189 ± 0.011	0.297 ± 0.006	1.13 ± 0.03	18.0 ± 0.21	18.3 ± 0.83
R:FR = 1.4	486 ± 21*	645 ± 43	0.157 ± 0.006*	0.246 ± 0.009**	1.33 ± 0.04**	15.9 ± 0.39**	15.5 ± 0.19*

of normal R:FR leaves for adaxial and abaxial stomata, respectively (Table 2). Thus, the greater g_s of the high R:FR leaves probably resulted from the higher stomatal density and/or the greater size. The adaxial and abaxial stomatal indices of the high R:FR leaves were also greater than those of normal R:FR leaves (Table 2). This indicates that the higher R:FR ratio accelerated stomatal development. The increase in stomatal density on the adaxial surface caused a lower ratio of abaxial to adaxial stomata in the high R:FR leaves. These results mostly agree with previous reports in which an increased proportion of active phytochrome enhances stomatal development (Schoch *et al.* 1984, Boccalandro *et al.* 2009, Casson *et al.* 2009), but with a stronger response on the adaxial surface than on the abaxial surface (Boccalandro *et al.* 2009). In general, sun leaves have a higher stomatal density than shade leaves and the increased density of stomata may favor their increased CO_2-uptake particularly at a high irradiance (Lichtenthaler *et al.* 1981). The stomatal development in the high R:FR-leaves may be enhanced as result of response similar to acclimation to a high irradiance.

In the P_N-c_i curves for each treatment group, P_N of the high R:FR leaves was greater than that of the normal R:FR-leaves at the same c_i (Fig. 2). This indicates that non-stomatal factors also contributed to the greater P_N of the high R:FR leaves. LMA of the high R:FR leaves was 1.23 times that of the normal R:FR leaves (Table 2). The greater LMA was probably also responsible for the greater P_N, because there is a tight relationship between photosynthetic capacity and LMA (Poorter *et al.* 2009). V_{cmax} of the high R:FR-leaves was 1.21 times higher than that of the normal R:FR-leaves. Thus, the non-stomatal photosynthetic advantage was partly due to a greater amount and/or activity of Rubisco. WUE_i of the high R:FR leaves was lower than that of the normal R:FR leaves (Table 1), although P_N of the high R:FR leaves increased. This is due to 1.51 times higher g_s of the high R:FR leaves than of the normal R:FR leaves, which is a greater number than the ratio for P_N (1.19). In transgenic *Arabidopsis thaliana* with an impaired response to R:FR, Boccalandro *et al.* (2009) demonstrated that active phytochrome enhances photosynthesis at the expense of water-use efficiency. By using an artificial radiation that has modified the proportion of active phytochrome in *C. sativus*, we confirmed this previous finding.

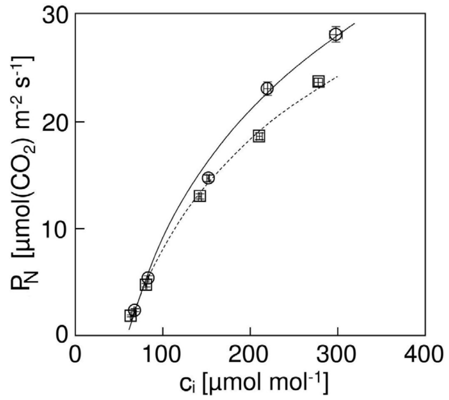

Fig. 2. Relationships between the intercellular CO_2 concentration (c_i) and the net photosynthetic rate rate (P_N) in *Cucumis sativus* first-true-leaves acclimated to irradiances with a high red:far-red (R:FR) ratio (= 10, the *circles*) or a normal R:FR (= 1.4, the *squares*) at PPFD of 2000 μmol(photon) m^{-2} s^{-1}. Ambient CO_2 concentrations were maintained at 75, 100, 200, 300, and 400 μmol mol^{-1}. Data are means ± SE of 10 replicate plants.

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