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BRIEF COMMUNICATION

Is the rate of photosynthesis under blue light altered in the *7B-1* tomato mutant?

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Abstract

Changes of photosynthesis under blue light were examined in the ABA-overproducing *7B-1* mutant in tomato. Net photosynthetic rate (P_N), stomatal conductance (g_s), intrinsic water-use efficiency (WUE_i) and chlorophyll ($a+b$) [Chl ($a+b$)] content in leaves of different insertion (1st, 4th and 9th ones) were measured in 5-, 7- and 9-week-old plants. P_N, g_s, and Chl ($a+b$) content were mostly similar in young leaves of *7B-1* and wild type (WT) plants. With the aging of leaves, a blue-light-induced increase in P_N and g_s to steady-state was delayed and steady-state values of P_N and g_s were lower in *7B-1* plants compared with WT. Steady-state values of WUE_i were increased in 4th and 9th leaves of *7B-1* plants compared with WT. The results can be explained by the higher endogenous level of ABA in *7B-1* plants and their lower sensitivity to ABA in earlier growth stage.

Additional key words: ABA-overproducer, chlorophyll content, net photosynthetic rate, *Solanum lycopersicum*, stomatal conductance, tomato mutant.

The *7B-1* mutant in tomato is an ABA-overproducer which has lower sensitivity to blue light in early ontogenetic stages: it shows reduced hypocotyl de-etiolation (Fellner *et al.* 2001). It has also lower blue-light-specific resistance of seed germination to osmotic stress (Fellner and Sawhney 2002). It is known that ABA causes stomatal closure (*e.g.* Assmann and Shimazaki 1999, Thompson *et al.* 2007) and that stomatal opening can be stimulated by blue light (*e.g.* Assmann and Shimazaki 1999, Košvancová-Zitová *et al.* 2009). Therefore we hypothesized that the *7B-1* mutant should have a reduced ability to open stomata which could result in a reduced rate of photosynthesis under blue light in comparison with WT. Thus the aim of this work was to find out whether and how the rate of photosynthesis under blue light was altered in *7B-1* plants.

Two genotypes of tomato (*Solanum lycopersicum* L.) were used in this study: cv. Rutgers (WT) and the *7B-1* mutant. Seeds of the *7B-1* mutant were kindly provided by Prof. Vipen K. Sawhney (University of Saskatchewan, Canada). Plants of both genotypes were grown during October 2010 in a greenhouse in soil (*Potgrond H*, *Klasmann Deilmann GmBH*, Germany) in small pots (80 × 80 × 70 mm, *i.e.* volume 448 cm^3; one seed per pot; 10 mm deep) and watered daily. High-pressure sodium lamps *PlantaStar E40/ES 400 W* (*Osram GmbH*, Germany) were used as a subsidiary light source to maintain a 16-h photoperiod. A temperature was adjusted from 15°C to 27°C. Three-week-old plants were transferred to a growth chamber *SGC.170.PFX.J* (*Weiss-Gallenkamp Ltd.*, Loughborough, UK) and let to acclimate for 2 weeks to stable growth conditions: the long-day

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Abbreviations: ABA – abscisic acid; Chl – chlorophyl; g_s – stomatal conductance; P_N – net photosynthetic rate; WT – wild type; WUE_i – intrinsic water-use efficiency.

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photoperiod (16 h light/8 h dark), photosynthetic photon flux density (PPFD) of about 100 µmol m^{-2} s^{-1} provided by fluorescent tubes *Luxline Plus F58W/830* (*Havells Sylvania GmbH*, Germany), a temperature of 25°C light/18°C dark and a relative air humidity of 50% light/55% dark. The plants were watered daily.

P_N was measured in fully expanded attached leaves as a rate of CO_2 assimilation using an open gas-exchange system *LI-6400* (*LI-COR Inc.*, Lincoln, NE, USA). The measurements were performed in three terms on leaves of different insertion when the plants were 5, 7, and 9 weeks old. 1st leaves of 5-week-old plants, 1st and 4th leaves of 7-week-old plants and 4th and 9th leaves (bearing the inflorescence) of 9-week-old plants were measured (leaves were counted from the plant base). Seven plants of the *7B-1* mutant and seven WT plants were used. The measurement was carried out during light period from 8:00 to 18:00 h in turns on WT and *7B-1* plants to eliminate effect of diurnal changes. The measured leaf was inserted into a leaf chamber and after 30-min adaptation to the dark and to the chamber conditions (*see* below) the measurement was started. The CO_2 concentration in the leaf chamber was set to 380 µmol mol^{-1}, the relative air humidity to 50% and the temperature of the air to 23°C. After 5 min of measurement in the dark, the leaf was exposed to blue actinic light (of about 380 µmol m^{-2} s^{-1}), P_N and g_s were measured for 65 min and then their time-response curves were drawn. The blue light was supplied by a source of cold white light (*Schott KL 2500*, *Schott Glas*, Wiesbaden, Germany) in a combination with a blue glass filter (*Schott BG 12*, *Schott Glas*, Wiesbaden, Germany) with thickness of 1.45 mm, dominant peak in 400 nm and spectral half width 132 nm. For P_N and g_s, steady-state values and time of steady-state achievement were evaluated. The time of steady-state achievement was estimated from an intersection of two lines of increasing and horizontal part of the time-response curve. WUE_i was calculated as a ratio of P_N and g_s values in steady-state.

Chl (*a*+*b*) content was estimated nondestructively by a chlorophyll meter *SPAD-502* (*Konica Minolta Sensing*, Japan) in the leaves used for the P_N and g_s measurement. The measurement was done 1 day after the gas-exchange measurement after 30 min of dark adaptation of plants. The SPAD reading was calibrated by means of an analytical measurement of real Chl (*a*+*b*) content in other tomato leaves. Discs of 14 mm in diameter have been cut off from the leaf blade at the site used for the SPAD measurement. The discs were frozen in liquid nitrogen, homogenized in 80% acetone with a small amount of $MgCO_3$ and centrifuged at 3,600 × *g* for 5 min. The Chl (*a*+*b*) content in the supernatant was determined spectrophotometrically (*Unicam UV550 Thermo Spectronic*, Cambridge, UK) with spectral slit width of 1 nm according to Lichtenthaler (1987). A linear correlation (r^2 = 0.955, $p<0.0001$) was found between the SPAD reading and leaf Chl (*a*+*b*) content:

$$\text{Chl } (a+b)\ [\mu\text{g cm}^{-2}] = 5.48 + 1.02 \times \text{SPAD reading}.$$

The two-sample *t*-test was used for comparison of presented parameters of the *7B-1* mutant and WT. The two-sample paired *t*-test was used for comparison of presented parameters of the same leaf (1st and 4th leaves) of different age. The statistical analyses were done using an *OriginPro 8.5.1* (*OriginLab Corporation*, Northampton, MA, USA).

When the dark-adapted leaves were exposed to blue light, P_N increased to a steady state (Fig. 1). Compared with WT, the *7B-1* mutant had a slower light-induced increase in P_N to the steady state in 1st leaf in 7-week-old plants and in 4th leaves in both 7- and 9-week-old plants (Fig. 1, Table 1). The slowdown was more pronounced in 9-week-old plants (Table 1). Further, the *7B-1* mutant had significantly lower steady-state values of P_N only in 1st and 4th leaves of 7- and 9-week-old plants, respectively (Fig. 1). Similarly, g_s increase to the steady state was slowed down only in 1st and 4th leaves of 7- and 9-week-old *7B-1* plants, respectively, and, in comparison with WT, the steady-state values of g_s were lower in 1st leaves of 7-week-old plants and in 4th leaves of both 7- and 9-week-old plants of *7B-1* (Table 1). Again, the difference in the g_s steady-state values between WT and *7B-1* became more pronounced with an increasing age of 4th leaves. Thus the significant and/or more pronounced differences in P_N and g_s were found between WT and *7B-1* when the measured leaves were 2 weeks older.

It means that although the *7B-1* mutant is an ABA-overproducer (Fellner *et al.* 2001) and shows lower sensitivity to blue light (Fellner *et al.* 2001, Fellner and Sawhney 2002), which should reduce stomatal opening, we can not make a general conclusion that the *7B-1* mutant had lower P_N and g_s under blue light. Compared with literature the results could indicate that the decrease in the P_N and g_s steady-state values together with the slowdown of their increase to the steady state could be associated with a faster ontogeny of mutant's leaves. Chl (*a*+*b*) content was not lower in *7B-1* compared with WT (Table 1) but both rate of photosynthesis and g_s can decrease during leaf ontogeny even before Chl content starts to decline (*e.g.* Xu *et al.* 1997, Majer and Hideg 2012) and stomatal opening has been shown to be slowed down with increasing leaf age (*e.g.* Ögren and Sundin 1996).

Nevertheless we suggest that the main reason for the differences found between photosynthetic parameters of *7B-1* and WT can be just the increased endogenous ABA level in *7B-1* as indicated by an increase in WUE_i (Table 1). It is known that WUE_i rises with increasing endogenous ABA level (*e.g.* Zhang *et al.* 2004) including ABA-overproducing transgenic tomato plants (Thompson *et al.* 2007). An unchanged WUE_i in 1st leaves of the *7B-1* mutant could be explained by their lower sensitivity to

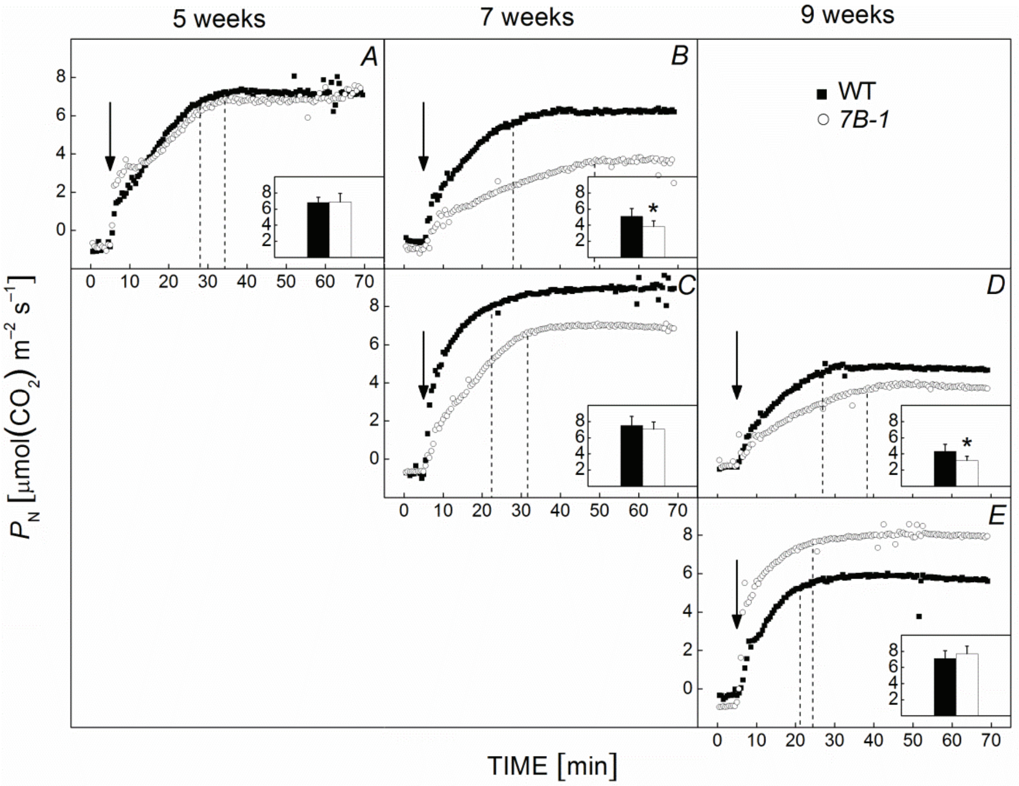

Fig. 1. Representative blue-light-induced increase in net photosynthetic rate (P_N) to steady-state in 1st leaf of 5-week-old plants (*A*), in 1st (*B*) and 4th leaves (*C*) of 7-week-old plants, and in 4th (*D*) and 9th (*E*) leaves of 9-week-old plants of wild type (WT) and the *7B-1* mutant. *Arrows* indicate switching the blue light on, *dashed lines* indicate time of steady-state achievement. *Insets*: steady-state values of P_N: black column – WT, white column – *7B-1*; means and SD are shown, n = 6–7; * significantly different ($p<0.05$).

Table 1. Time of net photosynthetic rate (P_N) and stomatal conductance (g_s) steady-state achievement, steady-state values of g_s, chlorophyll content [Chl (a+b)] and steady-state values of intrinsic water-use efficiency (WUE$_i$) in 1st (L1), 4th (L4) and 9th (L9) leaves of 5-, 7- and 9-week-old plants of wild type (WT) and the *7B-1* mutant. Means ± SD (n = 6–7). * significantly different ($p<0.05$) from the corresponding leaf of WT, $^+$ significantly different ($p<0.05$) from the leaf of the same insertion measured on the same type of plants two weeks earlier.

	5 weeks	7 weeks		9 weeks	
	L1	L1	L4	L4	L9
Time of P_N steady-state achievement [min]					
WT	28 ± 11	27 ± 4	21 ± 2	24 ± 4	21 ± 2
7B-1	34 ± 7	49 ± 6^{*+}	31 ± 6*	37 ± 8*	24 ± 9
Time of g_s steady-state achievement [min]					
WT	36 ± 6	39 ± 3	38 ± 7	34 ± 3	35 ± 4
7B-1	37 ± 7	55 ± 7^{*+}	43 ± 6	50 ± 7*	39 ± 8
g_s [mol(H$_2$O) m^{-2} s^{-1}]					
WT	0.13 ± 0.01	0.11 ± 0.02^{+}	0.22 ± 0.05	0.14 ± 0.04^{+}	0.25 ± 0.04
7B-1	0.14 ± 0.02	0.08 ± 0.02^{*+}	0.16 ± 0.04*	0.08 ± 0.01^{*+}	0.21 ± 0.06
Chl (a+b) [μg cm^{-2}]					
WT	36 ± 1	35 ± 2^{+}	36 ± 2	35 ± 4	44 ± 4
7B-1	36 ± 1	34 ± 4	37 ± 2	35 ± 2^{+}	47 ± 3
WUE$_i$ [μmol mol^{-1}]					
WT	51 ± 2	49 ± 12	35 ± 4	32 ± 7	29 ± 3
7B-1	50 ± 6	48 ± 13	47 ± 13*	42 ± 6*	39 ± 10*

endogenous ABA as young *7B-1* plants are supposed to be less sensitive to endogenous ABA (Fellner and Sawhney 2002).

We can conclude that the blue-light-induced increase in P_N and g_s to steady state was delayed and steady-state values of P_N and g_s were lower only when the measured leaves of *7B-1* were older and that these alterations in photosynthesis are most probably associated with the higher endogenous ABA level in *7B-1* plants. A measurement of more detailed dependence of P_N and g_s on leaf insertion and age could contribute to better understanding of peculiarity of *7B-1* photosynthesis.

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