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Spontaneous mutation *7B-1* in tomato impairs blue light-induced stomatal opening

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ABSTRACT

It was reported earlier that *7B-1* mutant in tomato (*Solanum lycopersicum* L.), an ABA overproducer, is defective in blue light (BL) signaling leading to BL-specific resistance to abiotic and biotic stresses. In this work, we examine responses of stomata to blue, red and white lights, fusicoccin, anion channel blockers (anthracene-9-carboxylic acid; 9-AC and niflumic acid; NIF) and ABA. Our results showed that the aperture of *7B-1* stomata does not increase in BL, suggesting that *7B-1* mutation impairs an element of BL signaling pathway involved in stomatal opening. Similar stomatal responses of *7B-1* and wild type (WT) to fusicoccin or 9-AC points out that activity of H^+-ATPase and 9-AC-sensitive anion channels *per se* is not likely affected by the mutation. Since 9-AC restored stomatal opening of *7B-1* in BL, it seems that 9-AC and BL could block similar type of anion channels. The stomata of both genotypes did not respond to NIF neither in darkness nor in any light conditions tested. In light, 9-AC but not NIF restored stomatal opening inhibited by ABA in WT and *7B-1*. We suggest that in comparison to WT, the activity of S-type anion channels in *7B-1* is more promoted by increased ABA content, and less reduced by BL, because of the mutant resistance to BL.

1. Introduction

Male sterility in crop plants, spontaneous or induced, is a choice material for plant breeders for several reasons, including its use in backcrossing, interspecific hybridization, and in F1 hybrid seed production. Sensitivity of male sterile mutants to abiotic or biotic stresses limits their use in breeding programs [1]. In contrast, the resistance of male sterile mutants to various stresses will certainly improve practical applications, e.g. in the hybrid seed industry. In tomato (*Solanum lycopersicum* L.), a spontaneous recessive single gene mutant *7B-1* [2] is characterized by reduced de-etiolation of hypocotyl growth, tall stature of adult plant, elevated levels of endogenous abscisic acid (ABA) and chlorophyll, and reduced levels of gibberellins, auxin, ethylene and cytokinins [3–5]. The mutant shows reduced responsiveness to various abiotic stresses specifically in blue light (BL) conditions [6,7]. We further showed that the *7B-1* mutation confers a BL-specific lower sensitivity to coronatine from *Pseudomonas syringae* [8]. Current results revealed that the mutant has defects in phototropic responses and in chloroplast movements (Bergougnoux et al., unpublished data), and shows changes in stomatal conductance, photosynthetic rate and intrinsic water-use efficiency [9]. The pleiotropic nature of the *7B-1* mutation suggests that a basic element involved in a BL signaling pathway(s) is affected.

Blue light triggers various developmental and signaling responses, including induction of stomatal opening [10]. Absorption of BL activates phototropin receptor kinases (PHOT1 and PHOT2) [11,12] associated with the plasma membrane [13,14]. The signal activates the plasma membrane H^+-ATPase in guard cells [15,16] that results in H^+ extrusion across the membrane responsible for plasma membrane hyperpolarization which is proposed to drive the K^+ uptake through the voltage-gated inward-rectifying K^+ channels [17]. The accumulated K^+ decreases an osmotic potential of guard cell facilitating the water influx into guard cells leading to an increase of turgor pressure in guard cells and stomatal opening [17]. The guard cell plasma membrane H^+-ATPase can be as well activated by some fungal toxins e.g. fusicoccin (FC) [18].

Stomatal opening is also promoted by inhibition of S-type anion (Cl^-, $malate^{2-}$) channels in guard cells caused by the activation of phototropins by light absorption [19]. Another BL mechanism of stomatal opening involves photoreceptors cryptochromes (CRY1

Abbreviations: 9-AC, anthracene-9-carboxylic acid; ABA, abscisic acid; BL, blue light; FC, fusicoccin; NIF, niflumic acid; PAR, photosynthetically active radiation; RL, red light; SE, standard error; WL, white light; WT, wild type.

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and CRY2) [20], although, phototropins are mostly accepted as BL receptors triggering stomatal movement.

Compared to BL, red light (RL) is less efficient in the induction of stomatal opening [21]. It was proposed that responses of stomata to RL are of photosynthetic origin and that chlorophyll is responsible for stomatal responses to RL; for review see [22]. However, recent studies showed that the RL-induced stomatal opening is independent of the concurrent photosynthetic rate of the guard cells and that it involves phytochrome signaling [23–26].

Mechanism of stomatal closure consisting in opening of anion channels promoting membrane depolarization is strongly supported by the facts that anion channel blockers regulate stomatal movements [27], and the initial study showed that ion channel blockers play an important role in study of stomatal signaling pathway. It was shown that anion channel blockers anthracene-9-carboxylic acid (9-AC) and niflumic acid (NIF) block current through R-type and S-type anion channels of plasma membrane of *Vicia faba* L. guard cells [27–30]. According to [28], NIF inhibits reversibly R-type anion channels in plasma membrane of guard cells more than 9-AC, whereas S-type of anion channel is more inhibited by 9-AC than by NIF [31]. In *Arabidopsis thaliana* L., 9-AC was able to induce stomatal opening in the dark, whereas, application of NIF have not changed the stomatal aperture [32]. In addition, in *Commelina communis* L., stomatal opening induced by white light (WL) could be enhanced by 9-AC [30].

ABA produced during water stress may induce stomatal closure or inhibit stomatal opening (e.g. [17]). Binding of ABA to its receptor of PYR/PYL/RCAR proteins (for review see [33]) subsequently produces H_2O_2 and NO and finally activates the anion channels that drive stomatal closure. The activation of the anion channels results in plasma membrane depolarization, and stimulation of voltage-gated K^+ channels, which releases K^+ out of the guard cell. In BL, ABA could suppress stomatal opening by inhibiting the phototropin mediated phosphorylation of the H^+-ATPase and K^+ uptake by inhibiting the inward-rectifying K^+ channels and BL-induced inward K^+ currents ([34–37]; for review see [38–40]). Both 9-AC and NIF reversed ABA inhibition of stomatal opening in *V. faba* L. and *C. communis* L. [30]. In *A. thaliana* L. and *C. communis* L., 9-AC reversed an effect of ABA in light [30,32], whereas NIF could not reverse ABA-induced stomatal closing in *A. thaliana* L. plants [32].

Our previous studies on *7B-1* mutant suggesting that the mutation could affect phototropin signaling led us to address the following question: Does the *7B-1* mutant exhibit differential responses to BL in stomatal opening, and, if so, is this response affected by anion channel blockers and ABA? Thus the aim of this work was to explore the reactions of *7B-1* stomata to light and to determine the effect of *7B-1* mutation in stomata guard cells.

2. Materials and methods

2.1. Plant material

The seeds of spontaneous mutant *7B-1* in tomato (*S. lycopersicum* L.) and its corresponding wild type (WT, cv. *Rutgers*) [2,7] were sown into soil 10 mm deep. The soil (pH (H_2O) 5.5–6.5) was composed of weakly spread bright peat (H_2–H_5) and of deeply chilled dark peat (H_6–H_8). Enriched with fertilizer NPK 14:16:18, the soil was then mixed with rough sand. The pots (height 0.1 m, width 0.06 m) with the seeds were placed in a home-made growth chamber and were watered once with a nutrient solution containing 0.8 mM $Ca(NO_3)_2$, 2 mM KNO_3, 60 μM K_2HPO_4, 695 μM KH_2PO_4, 1.1 μM $MgSO_4$, 20 μM $FeSO_4$, 20 μM Na_2EDTA, 74 nM $(NH_4)_6Mo_7O_{24}$, 3.6 μM $MnSO_4$, 3 μM $ZnSO_4$, 9.25 μM H_3BO_3, and 785 nM $CuSO_4$ [41]. Only water was used for subsequent watering of growing seedlings. The plants were cultivated in controlled conditions at relative humidity 70%, at 22 °C/20 °C during day/night (8/16 h). White light (200 μmol m^{-2} s^{-1}, photosynthetically active radiation (PAR) coming from incandescent light source) was used to illuminate the plants in growth chamber. The light irradiance was measured with quantum radiometer Li-Cor 185A (Lincoln, NE, USA). For experiments, first fully developed leaves in 3–5 week-old plants were used and their leaflets were harvested at the end of the night period.

2.2. Epidermal strip experiments

The major veins were separated from a harvested leaflet and the rest of the leaflet was cut to small pieces (about 5 mm × 5 mm). Two randomly selected pieces were glued to a microscopic cover glass coated with a layer of low viscosity glue (Telesis 5, Pacoima, CA, USA). The pieces were facing the cover glass by the abaxial side. The upper cell layers were peeled off with an edge of a microscopic glass, so the abaxial epidermal cells only with viable stomata remained on the cover glass. On such prepared samples, the stomata were still able to move due to low viscosity of the glue. The cover glass with epidermal strips were floated by "adaxial" side up in Petri dishes containing 5 ml of the incubation solution (50 mM KCl with 10 mM MES, pH 6.0 (TRIS)). To standardize the initial state, the samples in Petri dishes were incubated in darkness at 24 °C for 30 min.

In order to study stomatal opening induced by light, the Petri dishes with samples were placed to a box (ca. 0.125 m^3) illuminated by WL (the total photon fluence rate 300 μmol m^{-2} s^{-1}, incandescent light) and incubated at 24 °C. A thermal effect of illumination on samples (warming) was reduced by placing the Petri dishes with samples on the surface of flowing water. Blue and red lights were provided by covering the Petri dishes with color Supergel filters (Rosco Laboratories, Stamford, CT, USA). Blue filter no. 65 was used to provide BL of 60 μmol m^{-2} s^{-1}, and red filter no. 26 was selected to provide RL of 50 μmol m^{-2} s^{-1}. To investigate responses of dark-adapted stomata to FC, anion channels blockers 9-AC and NIF, the effectors were added to the Petri dishes with the samples before the illumination and the samples were incubated for 3 h. To investigate effects on light-adapted stomata, the effectors (9-AC, NIF or ABA [(±)-cis, trans-ABA]) were added to the samples 3 h after beginning of the illumination. The samples were then placed under the same light for subsequent 2.5 h. The anion channels blockers and FC were used in concentration of 100 μM and 10 μM, respectively. A sufficient ABA concentration inducing full stomatal closure was 1 μM. The dark-adapted samples were kept in the dark during all experimental time and stomatal aperture was measured at the end of experiment.

The microscopic cover glass with samples was pulled out from Petri dish and placed on a microscopic slide resulting into a sample together with a drop of the incubating solution between cover glass and the slide. Stomatal aperture was measured with an optical microscope (Nikon, Tokyo, Japan) fitted with a camera lucida and a digitizing table Calcomp Drawing slate II (Houston Instrument, Austin, TX, USA) connected with a personal computer as described in [42]. The sample was illuminated by very weak white light. Sixty stomatal apertures were measured during about 5 min in each sample and each condition. The mean and error bars (corresponding to a standard error (SE) calculated from several independent measurements) are showed in figures. Number of independent measurement is stated in figure legends.

Stomatal density (defined as number of stomata per mm^2) and stomatal index [defined as number of stomata/(number of stomata + number of epidermal cells)] was computed from microscopic pictures taken with the same microscopic objective magnification, dimensions of pictures were estimated using microscopic measure.

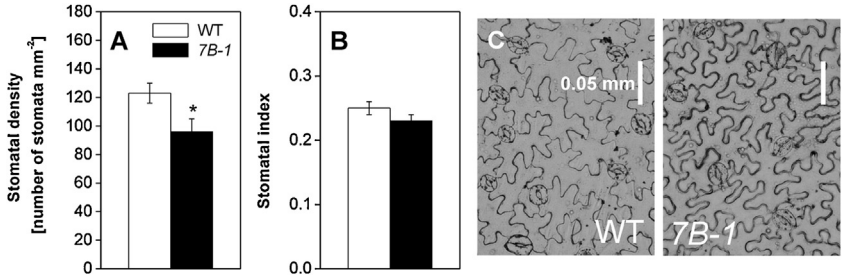

Fig. 1. Stomatal density (defined as number of stomata in mm^2) (A), stomatal index [defined as number of stomata/(number of stomata + number of epidermal cells)] (B) of WT (white column) and *7B-1* (black column) samples. Arithmetic means ± SE are shown, a number of evaluated samples was 21. Statistically significant difference between WT and *7B-1* is marked with an asterisk (*t*-test; $P < 0.05$). Microscopic picture (C) of WT and *7B-1* leaves shows the dimensions (white segment is a measure) and arrangement of cells.

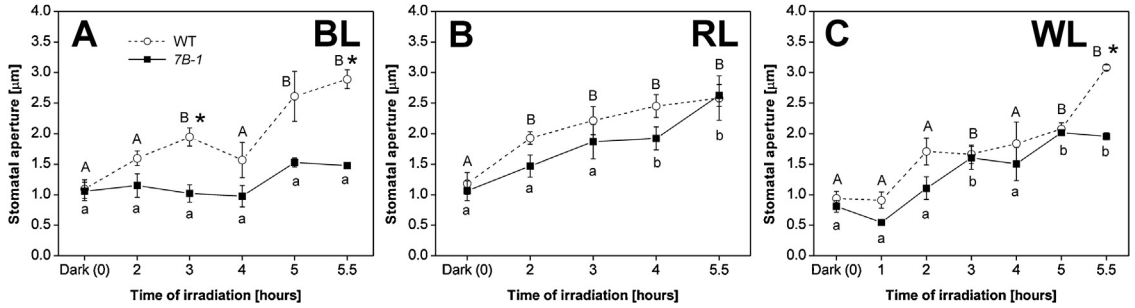

Fig. 2. Time course of stomatal aperture of WT (white dots and dash lines) and *7B-1* (black squares and solid lines) mutant in reaction (A) to blue light (BL, 60 μmol m^{-2} s^{-1}), (B) to red light (RL, 50 μmol m^{-2} s^{-1}) and (C) to white light (WL, 300 μmol m^{-2} s^{-1}). Arithmetic means ± SE of 3 independent experiments are shown (60 stomata per condition and per experiment). Letters A, B indicate significantly different groups for WT, letters a, b indicate significantly different groups for *7B-1*; asterisks indicate significant differences between WT and *7B-1* in one specific condition (*t*-test or Mann-Whitney rank sum test, see Section 2.3; $P < 0.05$).

2.3. Statistical analysis

The statistical differences were tested using *t*-test or Mann-Whitney rank sum test depending on the statistical properties of the data. Mann-Whitney rank sum test was used when the data did not have normal distributions or did not have the same variances. *P*-value of the applied test was compared with the critical value, which was chosen as 0.05. There was statistically significant difference between data if $P < 0.05$. Statistical software SigmaStat (Systat, Chicago, USA) version 3.0 was used for the testing.

3. Results

3.1. Stomatal density, stomatal index

Stomatal density of WT was significantly higher than that of *7B-1* (Fig. 1A), however, stomatal index (Fig. 1B) corresponding to percentage fraction of stomata among all the cells was similar in both genotypes. Despite the different arrangement and size of epidermal cells within *7B-1* and WT, stomata of both genotypes were of the similar size and surrounded by similar number of epidermal cells (Fig. 1C).

3.2. Responses of stomata to BL, RL and WL

In the dark, the stomatal aperture in *7B-1* mutant was similar to that observed in WT leaves. BL induced stomatal opening in WT leaves, whereas the *7B-1* stomata were insensitive to BL (Fig. 2A). In *7B-1* mutant, normal stomatal opening was induced only by RL and the aperture was comparable to that observed in WT plants (Fig. 2B). In WL, the mutant stomata opened less than in WT, but significantly only after 5.5 h of WL exposure (Fig. 2C).

3.3. Responses of stomata to FC

In order to show whether the extent of activation of guard cell plasma membrane H^+-ATPases differs between WT and *7B-1*, the responses of dark-adapted stomata of both genotypes to FC were

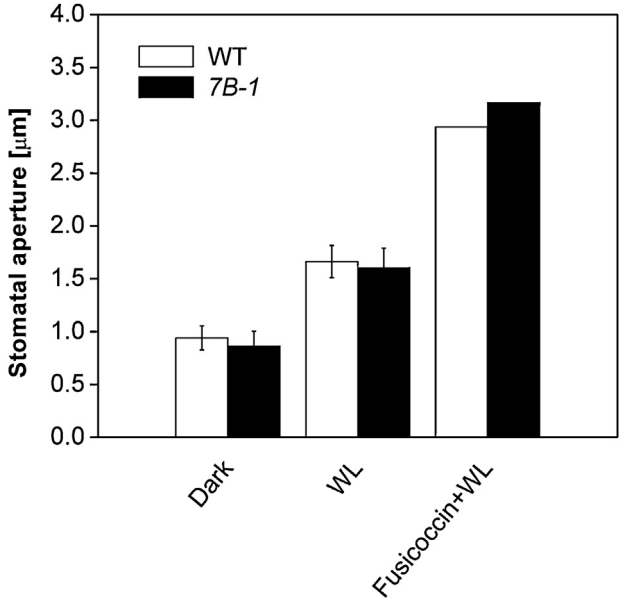

Fig. 3. Aperture of dark-adapted stomata of WT (white column) and *7B-1* (black column) and aperture of stomata non-treated or treated by fusicoccin before 3 h lasting exposition to white light (300 μmol m^{-2} s^{-1}) illumination (WL or fusicoccin + WL respectively). Arithmetic means ± SE of 3 independent experiments (1 in case of fusicoccin + WL) are shown (60 stomata per condition and per experiment).

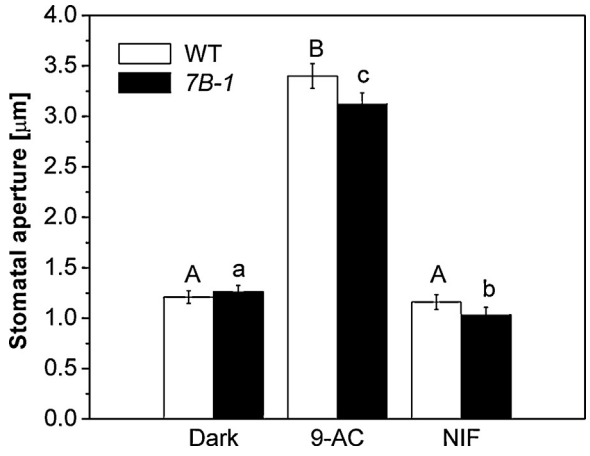

Fig. 4. Reaction of dark-adapted stomata of WT (white column) and *7B-1* (black column) tomato mutant to 3 h long action of ion channel blockers 9-AC (100 μM) and NIF (100 μM) in dark. Arithmetic means ± SE of 8 independent experiments are shown (60 stomata per condition and per experiment). Letters A, B indicate significantly different groups for WT, letters a, b indicate significantly different groups for *7B-1*. (*t*-test or Mann-Whitney rank sum test, see Section 2.3; $P < 0.05$).

compared (Fig. 3). Stomata treated by FC and/or exposed to WL considerably opened similarly in WT and *7B-1* leaves.

3.4. Stomatal responses to anion channel blockers

Adding anion channel blocker 9-AC (100 μM) to dark-adapted epidermal strips induced stomatal opening in both genotypes in a similar extent (Fig. 4). The effect of 9-AC was also observed on stomata of both genotypes pre-exposed (before 9-AC application) for 3 h by BL (RL or WL respectively) and afterwards exposed to BL (RL or WL respectively) for 2.5 h (Fig. 5A–C). Unlike 9-AC, NIF (100 μM), another anion channel blocker tested, did not stimulate stomatal opening in the dark in both genotypes (Fig. 4). When the stomata of WT pre-exposed by BL (RL or WL respectively) for 3 h were treated by NIF and subsequently exposed to BL (RL or WL respectively) for 2.5 h, the stomatal aperture was reduced (Fig. 5A–C). Since the stomatal aperture of *7B-1* under BL is low, the effect of NIF on stomata under BL was not considerable (the stomata remained almost closed, Fig. 5A). When the stomata of *7B-1* pre-exposed by RL (or WL respectively) for 3 h were treated by NIF and subsequently exposed to RL (or WL respectively) for 2.5 h, the stomatal aperture was reduced (Fig. 5B and C) as in case of WT.

3.5. Effects of anion channel blockers on ABA-induced stomatal closure

ABA was used as a factor that in contrast to light promotes stomatal closure or inhibits stomatal opening. Different responses to ABA in WT and ABA-overproducing *7B-1* [3] were expected. Stomatal opening in WT leaves induced by BL, RL or WL was completely inhibited by adding 1 μM ABA to the samples (Fig. 5A–C). Like in WT, ABA closed stomata in *7B-1* samples exposed to RL or WL (Fig. 5B and C). However, since BL is not capable to open stomata in *7B-1* mutant, no effect of ABA on stomata of the mutant could be seen (Fig. 5A).

The degree of reversibility of ABA-induced stomatal closing by anion channel blockers (9-AC and NIF) was tested. Fig. 5A–C shows that in BL, RL or WL, respectively, the anion channel blocker 9-AC restored the stomatal opening inhibited by ABA in both genotypes. Whatever the light conditions were, the stomatal closure induced by ABA in WT and *7B-1* mutant could not be overcome by blocker NIF (Fig. 5A–C).

4. Discussion

It was reported earlier that the tomato mutant *7B-1* shows a defect in BL signaling leading to resistance to various abiotic and biotic stresses specifically on BL [7,8]. Our current physiological and molecular studies suggest that this mutation could affect phototropin signaling (Bergougnoux et al., unpublished data). These facts led us to the question whether *7B-1* mutant exhibits different reactions to BL in stomatal responses.

In this work, we show that specifically in BL, *7B-1* has a defect in light induced stomatal opening. In our BL conditions, *7B-1* stomata are almost resistant to the stimulatory effect of the BL (Fig. 2A). The data are consistent with our previously published results. We earlier reported that 20-day-old *7B-1* seedlings show limited transpiration [3]. Recently, we demonstrated that in 5-week-old seedlings stomatal conductance in *7B-1* is lower than in WT [9].

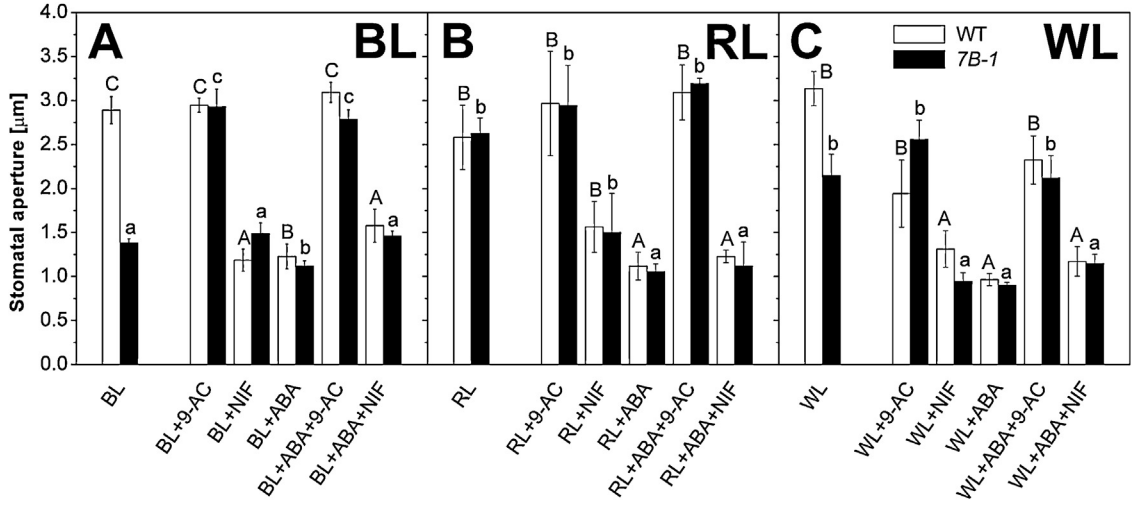

Fig. 5. Reaction of stomata of WT (white columns) and *7B-1* (black columns) tomato mutant illuminated for 3 h with (A) blue light (BL, 60 μmol m^{-2} s^{-1}), (B) red light (RL, 50 μmol m^{-2} s^{-1}) or (C) white light (WL, 300 μmol m^{-2} s^{-1}) to anion channels blockers 9-AC (100 μM) and NIF (100 μM), to ABA (1 μM) and to ABA (1 μM) together with the anion channels blockers 9-AC (100 μM) and NIF (100 μM). After adding the effectors, the samples have been put back under (A) BL, (B) RL or (C) WL for subsequent 2.5 h. Arithmetic means ± SE of 3 independent experiments are shown (60 stomata per condition and per experiment). Letters A, B, C indicate significantly different groups for WT, letters a, b, c indicate significantly different groups for *7B-1* (*t*-test or Mann-Whitney rank sum test, see Section 2.3; $P < 0.05$).

Our results could suggest that *7B-1* mutation impairs an element of phototropin signaling pathways within guard cells. To get more information about this defect, we further studied the *7B-1* stomatal responses to FC, two anion channel blockers 9-AC and NIF, and to ABA.

Fusicoccin has been found to activate H^+-ATPase in plasma membrane of guard cells [18], one of the processes involved in the light-induced stomatal opening. Similar stomatal responses of *7B-1* and WT to FC point out that the extent of activation of H^+-ATPase is probably not impaired by the *7B-1* mutation. This result also demonstrates that the function of H^+-ATPase itself is intact in *7B-1*.

In *Arabidopsis*, it was demonstrated that the anion channel blocker 9-AC induces stomatal opening in the dark [32]. In agreement with that, here we observed the similar stimulatory effect of 9-AC on stomata in both WT and *7B-1*, indicating that the function of 9-AC-sensitive anion channels *per se* is not likely impaired in *7B-1* stomata. Since the 9-AC blocks the S-type anion channels involved in stomatal opening [30] and we observed that in WT the BL did not further increase the stomatal aperture promoted by 9-AC (Fig. 5A), it seems that 9-AC and BL block the similar type of anion channels. This result is supported by observed slight 9-AC-induced increase of stomatal aperture under RL (Fig. 5B). Interestingly, although containing blue and red parts of spectra, WL in combination with 9-AC promoted stomatal opening less than BL (or RL) in combination with 9-AC in both genotypes. The explanation of this result is not clear. However, it differs from the result obtained on *C. communis* L., where stomatal opening induced by WL could be enhanced by 9-AC [30].

The second anion channel blocker NIF acted differently on stomatal movement as compared to 9-AC, and this was observed in both genotypes tested. The stomata did not respond to NIF in darkness. This is in accordance with the results of Forestier et al. [32] on *A. thaliana* L. plants. It suggests that NIF inhibits anion channels in plasma membrane that are different from those inhibited by light (S-type). It is also possible that NIF inhibits the Vcl channels in tonoplasts of vacuoles [43]. Also, NIF prevented stomatal opening in any light conditions tested in our experiments. This leads us to a hypothesis that NIF inhibits those anion channels working opposite to the anion channels inhibited by light (S-type).

Taken together, presented data contribute to our hypothesis that *7B-1* is impaired in early BL signaling pathway for stomatal opening (i.e. in signaling component(s) preceding the inhibition of S-type of anion channel in plasma membrane of guard cell). It is well known that stomata close in the presence of ABA and that ABA inhibits stomatal opening (e.g. [17]). We showed in several reports that *7B-1* is an ABA overproducer [3,8,44], while we demonstrated that ABA amount increases especially in BL-grown plants and that BL-induced accumulation of ABA was significantly higher in *7B-1* than in WT [8,44]. Therefore, stomatal opening of *7B-1* in BL may be inhibited by increased endogenous ABA content in *7B-1*. However, in several systems, we also reported that *7B-1* is less sensitive to BL in various responses, e.g. seed germination and hypocotyl elongation [5,7,8,44]. Currently, we also revealed reduced responses of *7B-1* specific for phototropins, such as phototropism, chloroplast movement, and early stage of BL-induced inhibition of hypocotyl elongation (Fellner et al., unpublished results). We therefore suggest that reduced stomatal aperture in *7B-1* in BL reflects, on the one hand, elevated level of ABA in *7B-1* mutant (i.e. increased stimulation of S-type anion channels), and reduced sensitivity to BL mediated by phototropin signaling pathway on the other hand (i.e. reduced inhibition of S-type anion channels) (Fig. 6). We still do not have direct evidence that *7B-1* has a defect in receptor PHOT1 or PHOT2. But since PHOT1 and PHOT2 functionally cooperate in BL responses (phototropism, chloroplast movement, stomata opening) [45], it is possible that the defect in one of the phototropin receptors can affect final responses regulated by the second phototropin receptor.

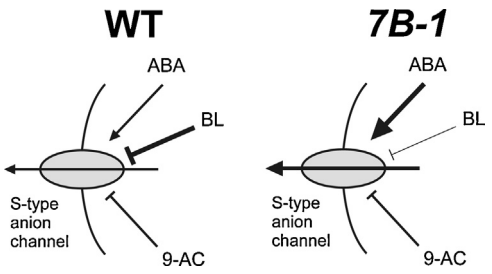

Fig. 6. Model explaining differential responses of WT and *7B-1* stomata to ABA and blue light (BL) and similar responses to anion channel blocker 9-AC. Our data suggest that reduced stomatal aperture in *7B-1* mutant is a consequence of elevated ABA content and reduced BL signaling in *7B-1* mutant. In addition, similar responses of WT and *7B-1* to 9-AC also indicate that ABA, BL and 9-AC take action likely in the similar type of anion channels. *Arrows* and *T-bars* represent positive and negative effects, respectively.

Further investigation of ABA effect on stomata showed that a relatively low concentration of exogenously added ABA (1 μM) inhibits stomatal opening (Fig. 5A–C) independently on light conditions. Under WL, the stomata of WT were more sensitive to endogenously applied ABA than the stomata of *7B-1* (Fig. 5C). Because of the insensitivity to BL, the sensitivity of *7B-1* to ABA is not obvious in our experimental protocol. However, we previously reported that the *7B-1* mutant shows BL-specific resistance to ABA [7].

In this work we further showed that in WT and at all light conditions tested, 9-AC restored stomatal opening inhibited by ABA. These results correspond to the results of Schwartz et al. [30] and Forestier et al. [32] obtained with *C. communis* L. and *A. thaliana* L. They confirm that in tomato there is a competition between a signaling component activated by ABA, which causes an activation of anion channel [38], and by 9-AC (or light) which causes an inhibition of anion channel. The full restoration of stomatal opening by 9-AC was also observed in *7B-1*. The 9-AC-induced restoration of stomatal opening in BL in the presence of ABA was of similar extent in WT and *7B-1* samples. It indicates that ABA, BL and 9-AC take action in the similar type of anion channels (Fig. 6).

In accordance to our results, minimal effect of NIF on ABA-induced stomatal closing was also observed by [32] in *A. thaliana* L. plants. It supports our presumption that NIF inhibits anion channels working opposite to those inhibited by 9-AC. Since in our experiments NIF always showed negative effect on stomatal opening, it is also possible that the anion channel blocker NIF in the concentrations used is toxic for tomato stomata.

Our experiments revealed that additionally to previously reported BL-specific characters, *7B-1* is also insensitive to BL-induced stomatal opening. Using FC, anion channel blockers and ABA suggests that the *7B-1* mutation affects signaling functioning in the inhibition of anion channels in plasma membrane of guard cells that is involved in stomatal opening. Our data indicate that previously reported increased endogenous ABA content in *7B-1* along with reduced mutant responses to BL is likely responsible for inhibition of stomatal opening in BL. In other projects, we currently endeavor to show that *7B-1* mutant has a defect in BL-receptor phototropin.

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