Journal of Experimental Botany Advance Access published February 12, 2009

Journal of Experimental Botany, Page 1 of 12
doi:10.1093/jxb/ern366

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

The *7B-1* mutation in tomato (*Solanum lycopersicum* L.) confers a blue light-specific lower sensitivity to coronatine, a toxin produced by *Pseudomonas syringae* pv. *tomato*

Véronique Bergougnoux[1], Vladimíra Hlaváčková[2], Renáta Plotzová[1], Ondřej Novák[3] and Martin Fellner[1,3,*]

[1] Palacky University in Olomouc, Faculty of Science, Department of Cell Biology and Genetics, Laboratory of Molecular Physiology, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

[2] Palacky University in Olomouc, Faculty of Science, Department of Experimental Physics, Laboratory of Biophysics, Tř. Svobody 26, 771 46 Olomouc, Czech Republic

[3] Palacky University in Olomouc, Faculty of Science, and Institute of Experimental Botany, Academy of Science of the Czech Republic, v.v.i., Laboratory of Growth Regulators, Šlechtitelů 11, 783 71 Olomouc, Czech Republic

Received 28 October 2008; Accepted 22 December 2008

Abstract

The spontaneous mutant *7B-1* in tomato (*Solanum lycopersicum*=*Lycopersicon esculentum*) is a photoperiod-dependent male-sterile mutant previously reported as resistant to various abiotic stresses specifically under blue light. Since this finding improved the potential of *7B-1*'s use in breeding programmes, its susceptibility to stress induced by coronatine (COR), the phytotoxine produced by several *Pseudomonas syringae* strains, was assessed in this study. The *7B-1* mutant was found to be less sensitive than the corresponding wild type (WT) to COR treatment in a blue light-dependent manner. Treatment of WT and *7B-1* plants with COR induced a strong accumulation of salicylic acid (SA), jasmonic acid (JA), and abscisic acid (ABA) in hypocotyls. Interestingly, accumulation of ABA and SA in the *7B-1* mutant was distinctly greater than in WT, especially in blue light. Based on the cross-talk between SA- and JA-signalling pathways, expression analysis of *NPR1* and *COI1* genes, respectively involved in these pathways, was investigated in COR-stressed plants. The blue light-specific lower sensitivity of *7B-1* plants to COR was found to be associated with blue light-specific overexpression of the *NPR1* gene. These data suggest that the SA-dependent NPR1-dependent pathway could be involved in the lower sensitivity of the *7B-1* mutant to COR. The role of anthocyanins and ABA accumulation during the response to COR is also discussed in the present study.

Key words: Blue light-specific response, *COI1*, coronatine, growth, *NPR1*, SA-signalling pathway, *7B-1* mutant, tomato (*Solanum lycopersicum* L.), *Lycopersicum esculentum* L.

Introduction

Male sterility of crop species, spontaneous or induced, is a criterion of importance for breeders. In almost all crops, several male-sterile mutants have been isolated. Nevertheless, their use in breeding programmes has been limited as some of them were sensitive to abiotic stress, mainly temperature and drought (for review, see Kaul, 1988; Rao *et al.*, 1990). The resistance of male-sterile mutants to abiotic stress, as well as to biotic stress, should greatly increase their value with regard to practical applications, such as in the hybrid seed industry. The tomato (*Solanum lycopersicum=Lycopersicon esculentum* L.) is one of the most important crop species worldwide, with a production exceeding 125 million tons in 2006 (source: Food and Agriculture Organization of the United Nations, 2008; http://www.fao.org/). In the late 1990s, a recessive single gene mutant, *7B-1*, was isolated based on its photoperiod-dependent male-sterility (Sawhney, 1997,

* To whom correspondence should be addressed. E-mail: martin.fellner@upol.cz

2004). Previous studies have demonstrated that the *7B-1* mutant was resistant to several abiotic stresses in a specific blue light-dependent manner (Fellner and Sawhney, 2001, 2002). It is well known that in some systems blue light is involved in cell osmoregulation (Marten *et al.*, 2007; for a review, see Shimazaki *et al.*, 2007). But the involvement of blue light in responses to abiotic stresses was demonstrated for the first time in the tomato (Fellner and Sawhney, 2002). The spontaneous *7B-1* mutant, defective in responsiveness to blue light, was used to study the role of light in sensitivity to coronatine (COR), a toxin produced by *Pseudomonas syringae*.

In order to protect themselves from pathogen attacks, plants in general have evolved sophisticated mechanisms. To induce effective resistance, it is absolutely necessary for plants to activate the specific signalling pathways that will be more likely to counteract the invader. At the same time, other signalling pathways not involved in pathogen resistance or with minimal effect will be attenuated or simply suppressed to avoid depletion of valuable physiological resources (Anderson *et al.*, 2007). In the response to pathogen attack, it is commonly accepted that the salicylic acid (SA)-signalling pathway and the jasmonic acid (JA)/ethylene-signalling pathway are antagonistic, while SA is involved in resistance against biotrophic pathogens and JA/ethylene is involved in resistance against necrotrophic pathogens (reviewed in Kunkel and Brooks, 2002; Anderson *et al.*, 2007). Antagonistic interactions between both pathways have been studied in different species, and particularly in *Arabidopsis*. Thus, in NahG *Arabidopsis* transgenic plants, unable to synthesize and accumulate SA, more genes were involved in the JA pathway compared with the corresponding wild type (WT), demonstrating that SA accumulation is associated with suppression of the JA-induced pathway (Spoel *et al.*, 2003). Furthermore, Spoel *et al.* (2003) demonstrated that *NPR1*, an important element in the SA-signalling pathway, controls the SA-induced suppression of the JA pathway. Inversely, in tobacco it was shown that JA inhibits the expression of SA-induced genes (Niki *et al.*, 1998). It is then conceivable that pathogens have evolved strategies based on antagonism to induce the efficient promotion of disease. Nevertheless, this antagonistic scheme between both pathways is oversimplified and recent studies tend to demonstrate that these two signalling pathways could act together to control a pathogen attack. Several studies have demonstrated that the response of plants to pathogen attacks is not limited to the stimulation of the two SA and JA hormones, as the induction of other phytohormones has been reported (Robert-Seilaniantz *et al.*, 2007). Thus, concomitantly, it was established that pathogen attacks induce auxin and abscisic acid (ABA) accumulation (O'Donnell *et al.*, 2003; Schmelz *et al.*, 2003).

Bacterial speck, caused by *Pseudomonas syringae* pv. *tomato* (*Pst*), is one of the diseases affecting tomato under field and greenhouse conditions (Zhao *et al.*, 2003). *Pst* can be considered as a hemi-biotrophic pathogen, as the most aggressive intercellular growth phase occurs without cell death (Nomura *et al.*, 2005). Among strategies developed to induce host colonization, *Pst* produces another virulence factor, the phytotoxin COR, which is indispensable to ensure total virulence of the bacteria in plant cells (Bender *et al.*, 1999; Nomura *et al.*, 2005). Although COR is not required to induce the pathogenicity of *Pst*, its production results in increasing the severity of the damage caused by the bacteria and contributes to systemic movement of bacteria *in planta*, promotion of lesion expansion, and multiplication of bacteria in host cells (Bender *et al.*, 1999; Zhao *et al.*, 2003). The symptoms induced by COR are intense chlorosis, hypertrophy, inhibition of root growth, stimulation of ethylene production, synthesis of anthocyanins, accumulation of alkaloids, and promotion of disease-associated senescence (Weiler *et al.*, 1994; Bender *et al.*, 1999; Zhao *et al.*, 2003; Uppalapati *et al.*, 2005). COR is a polyketide composed of coronafacic acid (Parry *et al.*, 1994; Rangaswamy *et al.*, 1998) and coronamic acid, a cyclized derivative of isoleucine (Mitchell, 1985). Both moieties of COR have structural analogies with endogenous plant signalling molecules. Whereas coronamic acid is similar to aminocyclopropyl carboxylic acid, the direct precursor of ethylene, and has biological activity *in planta*, it does not function as an analogue of aminocyclopropyl carboxylic acid (Uppalapati *et al.*, 2005). The coronafacic acid moiety is structurally and functionally similar to several jasmonates and, more particularly, the endogenous jasmonate JA-Ileu (Krumm *et al.*, 1995; Staswick and Tiryaki, 2004). Studies of COR-insensitive mutants in *Arabidopsis* (*coi1*; Feys *et al.*, 1994) and tomato (*jai1*; Zhao *et al.*, 2003) provided information about COR-induced pathogenicity. These mutants are JA-insensitive and accumulate higher amounts of SA, resulting in a higher resistance to *Pst*. Li *et al.* (2004) demonstrated that *JAI1* is a homologue of *COI1*. The *JAI1*/*COI1* gene encodes an F-box protein involved in ubiquitin-dependent protein degradation and determines the target specificity of the E3 ubiquitin ligase SCF^{COI1} (where SCF indicates Skp/Cullin/F-box; Xie *et al.*, 1998; Katsir *et al.*, 2008). This complex controls the degradation of jasmonate ZIM domain (JAZ) proteins that repress the transcription of the JA-responsive genes (Zhao *et al.*, 2003; Santner and Estelle, 2007; Katsir *et al.*, 2008). Recently, Thines *et al.* (2007) demonstrated that the physical interaction between COI and JAZ is strictly dependent on JA-Ileu. As COR has been shown to be a homologue to JA-Ileu, it is understandable that COR can also induce the COI/JAZ complex formation, thus inhibiting the JA-signalling pathway repression and allowing the establishment of pathogenicity (Krumm *et al.*, 1995; Staswick and Tiryaki, 2004).

Studies on resistance to biotic stress are mostly conducted on the model plant *Arabidopsis thaliana*. Conversely, little information is available about crop species. Using the spontaneous *7B-1* mutant affected in the blue light response, the sensitivity of tomato to COR-induced stress was studied. The question of whether and how blue light can directly affect the response to COR-induced stress was addressed. Whereas resistance to abiotic stress was assessed

based on seed germination of the spontaneous *7B-1* mutant, this paper focused for the first time on the response of young *7B-1* seedlings to biotic stress. These results constitute the first step towards understanding the role of blue light in plant responses to biotic stress.

Materials and methods

Plant material and growth conditions

The single-gene recessive *7B-1* mutant in tomato (*Solanum lycopersicum* L. background cv. Rutgers) was isolated as a photoperiod-sensitive male-sterile mutant (Sawhney, 2004). To obtain sterile cultures, seeds were soaked in Savo solution (3% sodium hypochlorite) (Bochemie, Czech Republic) for 20 min, rinsed extensively with sterile distilled water, and sown on 0.7% (w/v) agar medium in Petri dishes (ϕ 90 mm). The basal medium (BM) contained Murashige and Skoog salts (Murashige and Skoog, 1962), 1% (w/v) sucrose, and 1 mM MES [2-(*N*-morpholino)-ethanesulphonic acid], pH 6.1. Seed germination was induced in the dark for 4 d at 23 °C. Petri dishes with germinated seeds were placed in a culture chamber (Snijders, The Netherlands) at 23 °C, under continuous light. Fluorescent lamps were used to provide blue light (TL-D 36W/Blue, Phillips; total photon fluence rate 20 μmol m^{-2} s^{-1}) and red light (TL-D 36W/Red, Phillips; total photon fluence rate 10 μmol m^{-2} s^{-1}). Light spectra were measured using a portable spectroradiometer (model LI-1800; Li-COR, Lincoln, NE, USA). For dark conditions, Petri dishes were wrapped in aluminium foil, and placed in the same culture chamber under the same temperature regime.

Hypocotyl growth measurement

Germinated seeds were transferred on BM containing various concentrations of COR (Sigma-Aldrich, Czech Republic). Petri dishes were placed vertically in the culture chamber at 23 °C and the seedlings were incubated for 7 d under continuous light (blue or red) or in the dark. Hypocotyl length was measured with a ruler and the percentage of hypocotyl growth inhibition was determined. Three to six independent experiments were done for every treatment with at least 10 plantlets in each.

Determination of chlorosis and chlorophyll quantification

Seeds were sown in soil in the greenhouse. Folioles from 6–12 mature leaves of 3-week-old plants were inoculated with either water or 1 nmol of COR, contained in a 1.6 μl droplet. At 7 d and 15 d post-inoculation (dpi), leaves were detached from the plants and scanned. Chlorotic surfaces were determined using ImageJ software (Abramoff *et al.*, 2004) and expressed as a percentage of the whole leaf area. Inoculated folioles (7 and 15 dpi) were further used for quantitative chlorophyll determination. For this purpose, leaves were placed in *N*-*N*′-dimethylformamide (Sigma-Aldrich, Czech Republic) for 7 d in darkness at 4 °C. The extract was measured by spectrophotometer (model SmartSpec™ Plus; Bio-Rad, Czech Republic) at 664.5 nm (for chlorophyll *a*) and 647 nm (for chlorophyll *b*). Total chlorophyll content was determined according to Inskeep and Bloom (1985). Results were expressed as the ratio of chlorophyll content between treated and control folioles.

Anthocyanin determination

Anthocyanins were extracted as described by Sheoran *et al.* (2006) with little modification. Hypocotyls from plants grown on various concentrations of COR in dark or blue or red light were harvested, weighed, ground in a pre-cooled mortar with 1 ml of cold ethanol (96%) containing 1% (v/v) HCl, and centrifuged for 10 min at 20 000 *g* at 4 °C. Anthocyanin content was measured by spectrophotometer at 535 nm and calculated according to Sheoran *et al.* (2006). Interference with chlorophyll accumulation in light-grown hypocotyls was corrected by measuring absorbance at 640 nm. Results were expressed in nmol of anthocyanins per hypocotyl.

Analysis of COR-induced gene expression by quantitative-RT-PCR (q-RT-PCR)

Seedlings transferred on BM ±100 nM COR were placed vertically in a culture chamber at 23 °C and continuous dark, blue light, or red light. Five days after transfer, hypocotyls were harvested, immediately frozen in liquid nitrogen, and ground in liquid nitrogen using a mortar and pestle. The total RNA was extracted using an RNeasy Plant Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. An additional DNase I treatment was performed using RQ1 RNA-free DNase (Promega, USA) for 30 min at 37 °C, and purification of the RNA was performed by a subsequent phenol/chloroform/iso-amylalcohol (25:24:1) step. A reverse transcription reaction was performed from 1 μg of the total RNA by adding 4 μl of RT buffer, 1 μl of 10 mM dNTP, 1 μl of 0.1 M DTT, 1 μl of 50 μM oligo$(dT)_{15}$ primer, 20 units of RNasin Plus RNase inhibitor (Promega, USA), and 1 μl of SuperScript™ III reverse transcriptase (Invitrogen, USA). The volume was filled up to 20 μl using RNase-free water. The reaction mixture was then incubated at 50 °C for 60 min followed by heat inactivation of reverse transcriptase by incubation at 70 °C for 15 min. RNA was digested by 5 units of RNase H (New England Biolabs, USA) for 20 min at 37 °C. The cDNA was subsequently purified on a column and eluted in sterile RNase-free water before use for q-RT-PCR analysis. Amplification of target genes and real-time detection of amplicon production were monitored on an Mx3000P sequence detector (Stratagene, USA). q-RT-PCR reactions contained 80 nM of each primer, 5 μl of cDNA template (diluted 1/100), 12.5 μl of 2× absolute SYBR Green ROX Mix (ABGene, Epsom, UK). The volume was filled up to 25 μl with sterile RNase-free water. PCR cycling conditions required an initial HotStart activation of 15 min at 95 °C, followed by 45 cycles of 95 °C for 30 s and 60 °C for 30 s. To confirm the product purity, a melting curve

analysis was performed at the end of the PCR reaction and an aliquot of each PCR product was loaded onto a 3% agarose gel. The SYBR green fluorescent signal was standardized with a passive reference DYE (ROX) included in the SYBR green PCR master mix. The target gene expression was quantified relative to the expression measured from the reference gene in the same sample. Different reference genes were tested and a subsequent analysis by geNorm (Vandesompele *et al.*, 2002) determined that ubiquitin (accession number TC173965) was the best reference gene in the present working conditions. Differences in the cycle numbers during the linear amplification phase between the samples and the $\Delta\Delta C_T$ method were used to determine the differential gene expression. Gene expression in *7B-1* hypocotyls was expressed as relative to that obtained for WT hypocotyls grown in the dark in the absence of COR. The set of primers used in the expression of selected genes is given in Table 1.

Determination of ABA, JA, and SA

Seedlings were transferred on BM supplemented with 100 nM COR, placed in a growth chamber, and cultured for 7 d at 23 °C in dark or blue or red light. Control plantlets were grown on BM without COR. Hypocotyls were harvested and immediately frozen in liquid nitrogen. ABA, JA, and SA extraction and quantification were modified according to Hlavačková *et al.* (2006). Plant material was extracted overnight in 80% methanol. In each extract, 100 pmol of [$^2H_4$]SA (Cambridge Isotope Laboratories, Andover, MA, USA) and 50 pmol of [$^2H_6$]ABA and [$^2H_6$]JA (Olchemim Ltd, Czech Republic) were added as internal standards to check the recovery during purification and to validate the determination. The extracts were purified using a combination of an octadecylsilica column (500 mg; Agilent Technologies, Palo Alto, CA, USA) and a DEAE-Sephadex (Sigma Aldrich, Taufkirchen, Germany) with a Sep-Pak C18 cartridge (360 mg; Waters, Milford, MA, USA). The diethyl ether elutes from the C18 column were dried with nitrogen and stored at –20 °C until further analyses. The purified samples were quantified by the LC-MS/MS system consisting of an Alliance 2695 Separations Module (Waters) with Quatro micro API (Waters) triple quadrupole mass spectrometer equipped with an electrospray interface operated in the negative mode. The purified samples were dissolved in 10% acetonitrile and injected onto a Luna phenyl–hexyl column (250×2.0 mm, 5 μm; Phenomenex, Torrance, CA, USA). The column was eluted with a linear gradient (0 min, 10% B; 0–10 min, 50% B; 10–12 min, 80%; flow-rate of 0.25 ml min^{-1}) of 10% formic acid (A) and acetonitrile (B). Quantification was obtained by a multiple reaction monitoring (MRM) mode of $[M\text{-}H]^-$ and the appropriate product ion. The MRM transitions 269.2>159.1, 215.1>59, and 141.1>97 were used for labelling, and 263.2>153.1, 209.1>59, and 137.1>93 were used for authentic ABA, JA, and SA, respectively. For selective MRM experiments, optimal conditions were as follow: capillary/cone voltage, 1.0 kV/25 V; source/desolvation gas temperature, 100/350 °C; cone/desolvation gas, 2.0/500 L/h; LM/HM resolution, 12.5; collision energy, 15 eV; ion energy 1, 0.3 V; ion energy 2, 1.5 V; entrance, 2.0 V; exit, 2.0 V; multiplier, 650 eV. In MRM mode, the limit of detection (signal to noise ratio=3) for all three analytes was 100.0 fmol, and the linear range was 0.25–1000 pmol per injection with a correlation coefficient of 0.9990–0.9995. Three independent experiments were performed for each treatment.

Statistical analysis

The statistical significance of the treatment differences was assessed using Student's *t*-test.

Results

COR induces reduced symptoms in the 7B-1 *mutant* in planta

Control leaves of WT and *7B-1* genotypes were green without any sign of chlorosis independent of the duration of the experiment (7 or 15 dpi; data not shown). Conversely, chlorosis developed in both genotypes when the leaves were inoculated with 1 nmol COR (Fig. 1A). Although leaf surfaces undergoing chlorosis were almost the same in both genotypes at 7 dpi, chlorosis symptoms increased during the time lapse of the experiment in both genotypes, with WT plants developing essentially larger surfaces of chlorosis than *7B-1* plants (Fig. 1B).

Chlorophyll content was also monitored in WT and *7B-1* genotypes at 7 and 15 dpi, as an indicator of chlorosis and chlorophyll degradation. In untreated plants, chlorophyll content was higher in *7B-1* plants than in WT plants (data not shown), confirming previous results obtained by Fellner *et al.* (2005). After 7 d of COR treatment, no clearly visible differences could be seen in the chlorophyll content of WT and *7B-1*-treated leaves (Fig. 1C).

Table 1. Sequences of primer sets used for quantitative real-time PCR

Gene name and accession number	Primer sequence	Function
COI1 (AY423550) coronatine insensitive 1	Upper 5′-ATGGGCGAGCCATCGCTAAG	JA-signalling pathway
	Lower 5′-AGCCTTGGCTACCTTGCAGC	
NPR1 (AF143442) non-expressor of PR1	Upper 5′-GGCGGACAACCTGCGTCAAC	SA-signalling pathway
	Lower 5′-GCTCTCGTGGTCTGGCAAGC	
UBQ (TC173965) ubiquitin	Upper 5′-TTCCTTCAGACCAGCAGAGGTTGA	
	Lower 5′-TTTGCATACCACCACGTAGACGGA	

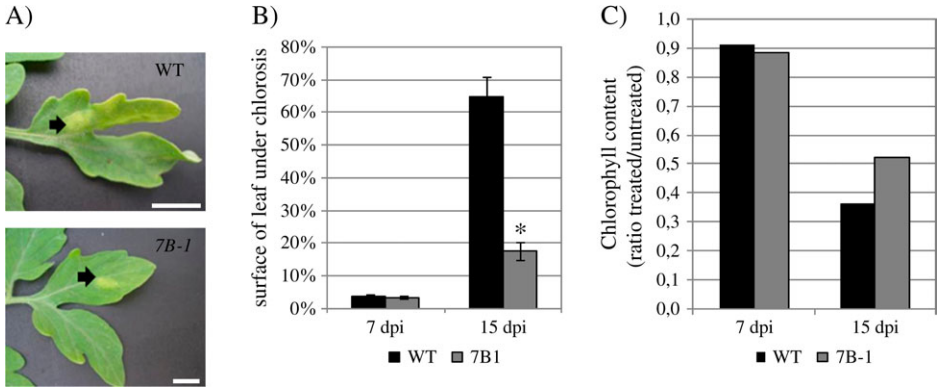

Fig. 1. Effect of coronatine (1 nmol) on leaves of WT and *7B-1* tomato mutant. (A) Chlorosis development 7 dpi. Scale bars represent 1 cm. (B) Estimation of leaf surface undergoing chlorosis 7 dpi and 15 dpi. Data are expressed as a percentage of the whole leaf undergoing necrosis. Results represent the average ±SE of 6–12 plants analysed. (C) Chlorophyll content in leaves 7 dpi and 15 dpi. Three to six plants were analysed for chlorophyll content and the average chlorophyll content was calculated for each condition and each genotype. Values represent the ratio between treated and untreated samples. * Significantly different from the WT ($P \leqslant 0.05$).

However, at 15 dpi, COR induced stronger degradation of chlorophyll in the WT (35% of chlorophyll left compared with the control) than in *7B-1* (52% of chlorophyll left compared with the control).

The lower sensitivity of the 7B-1 *mutant to high COR is blue-light specific*

In vivo experiments demonstrated that the *7B-1* mutant is less sensitive to COR in comparison with the WT. *In vitro* assays were developed to investigate further the response of the *7B-1* mutant to COR-induced stress. Seedlings of the WT and *7B-1* mutant were grown on various concentrations of COR (0.1–100 nM) and under different light conditions (dark, blue light, or red light). At the higher concentrations (10 nM and 100 nM) of COR in the culture medium, both the WT and *7B-1* seedlings showed chlorosis of cotyledons and a severe reduction in hypocotyl and root growth in a light-independent manner (Fig. 2A). At low concentrations of COR (0.1 and 1 nM), hypocotyl growth was weakly affected in both genotypes. At higher concentrations of COR (10 nM and 100 nM), WT and *7B-1* responded differently to COR depending on the light quality. Under blue light, growth of WT seedlings was more inhibited by COR than that of *7B-1* seedlings (Fig. 2B). Although hypocotyl growth in WT plants under dark and red light seemed to be more inhibited than that of the *7B-1* mutant, differences were found to be not significant.

At all the COR concentrations and light conditions, no significant differences in root responses were observed between both genotypes, but root growth was highly sensitive to COR as seedlings subjected to 1 nM COR showed strong inhibition (35%; data not shown).

One of the symptoms reported to be induced by COR is the accumulation of anthocyanins (Uppalapati *et al.*, 2005). Increasing concentrations of COR correlated with more purple colour in the hypocotyls of both WT and *7B-1* seedlings, especially at the base of the hypocotyl and

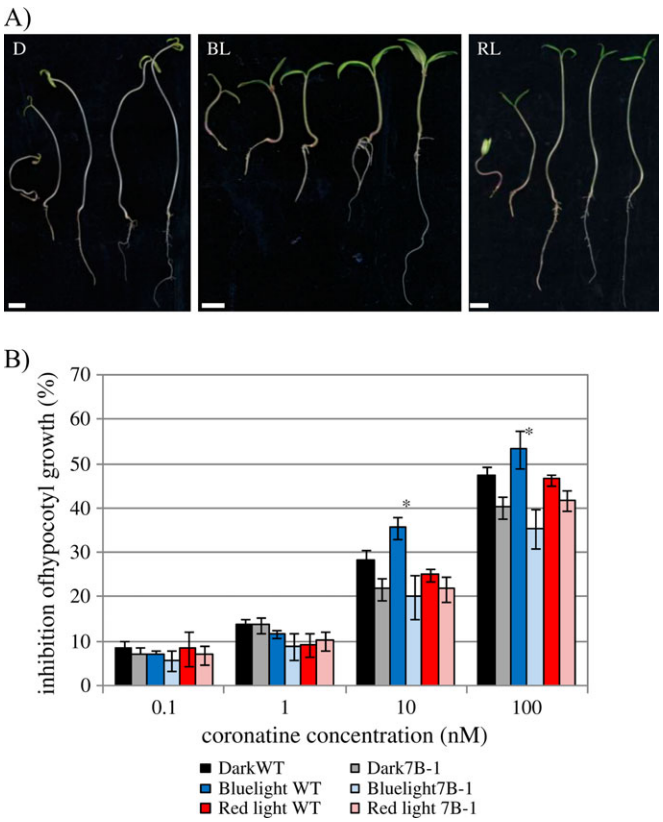

Fig. 2. Effect of various coronatine concentrations on WT and *7B-1* tomato growth after 7 d under different light qualities. (A) Phenotype of WT seedlings under decreasing coronatine stress (from left to right) in dark (D), blue light (BL), and red light (RL). Scale bars represent 1 cm. (B) Percentage of hypocotyl growth inhibition of WT and *7B-1*. Results presented are averages ±SE of four to six independent biological repeats. * WT and *7B-1* show a significant difference (*t*-test; $P \leqslant 0.05$).

above the cotyledons (Fig. 3A). It is known that anthocyanins accumulate under stress conditions. In order to evaluate possible differences in anthocyanin accumulation

in relation to COR-induced stress, anthocyanins were extracted and quantified from hypocotyls of both genotypes grown under various concentrations of COR, and in different light qualities. In untreated plants, accumulation of anthocyanins was higher in blue light than in dark and red light in both genotypes, with *7B-1* seedlings accumulating less anthocyanin than WT seedlings. When plants were subjected to increasing concentrations of COR, both genotypes accumulated anthocyanins to a great extent, independent of the light quality. Whereas no differences were observed between WT and *7B-1* in dark and red light, under blue light, *7B-1* accumulated significantly less anthocyanin than WT, especially at high COR concentrations (Fig. 3B).

Lower sensitivity of the 7B-1 *mutant to high COR correlates with increased accumulation of ABA and SA*

ABA, SA, and JA accumulations in the WT and *7B-1* plantlets, subjected to high COR concentration (100 nM), were investigated. As variability between the three experiments performed was important but trends were similar, data presented in Fig. 4 are results obtained from one experiment. Other data are shown in Supplementary Fig. S1 available at *JXB* online. In the absence of COR, *7B-1* hypocotyls accumulated a higher amount of ABA than WT plants, especially under blue light and red light in agreement with the fact that the *7B-1* mutant is an ABA-overproducer (Fellner *et al.*, 2001). COR treatment induced strong ABA

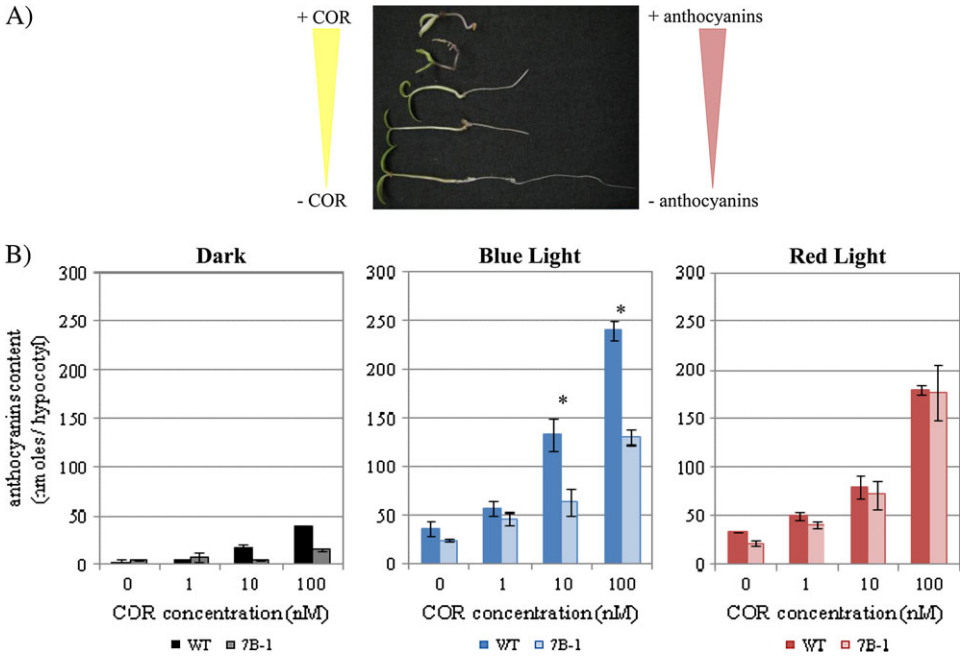

Fig. 3. Coronatine-induced anthocyanin content in 7-d-old hypocotyls. (A) Content of pigments in WT hypocotyl COR concentration-dependence. (B) Anthocyanin concentration (nmol hypocotyl^{-1}) in hypocotyls of WT and *7B-1* seedlings. Results presented are averages ±SE of three independent experiments. * WT and *7B-1* show a significant difference (*t*-test; $P \leqslant 0.05$).

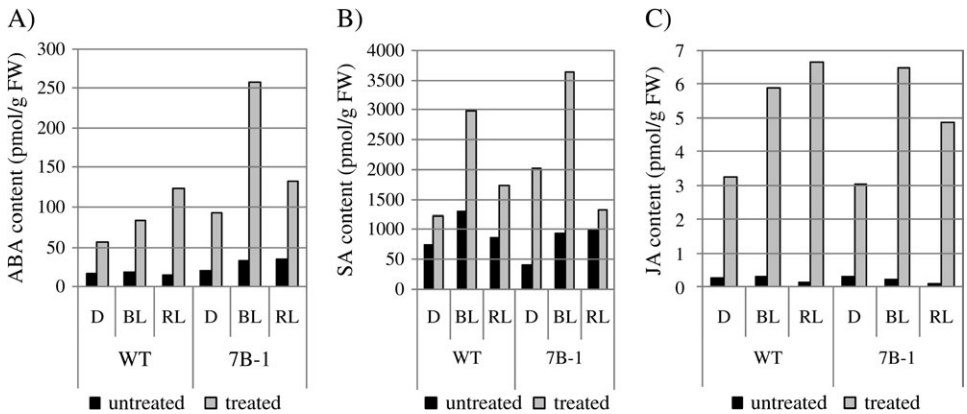

Fig. 4. Effect of COR treatment (100 nM) on hormone content in hypocotyls of WT and *7B-1* tomato seedlings grown for 7 d in dark (D), blue light (BL), and red light (RL): (A) ABA concentration; (B) SA concentration; (C) JA concentration. Three independent experiments were performed. The data shown are data obtained from one representative experiment.

accumulation in hypocotyls of both genotypes. ABA accumulation was more pronounced in *7B-1* hypocotyls than in WT (Fig. 4A), in particular under blue light.

In untreated plants grown in dark or blue light, SA content was markedly lower in *7B-1* than in WT hypocotyls. After a COR treatment, both genotypes accumulated SA to a greater extent than unstressed plants. Nevertheless, in dark and blue light, *7B-1* was much more efficient than WT in SA accumulation (Fig. 4B). Concentrations of JA in untreated plants of both genotypes were very small. After a COR treatment, JA content considerably increased in both genotypes, especially in light. However, unlike ABA or SA, the concentration of JA in the hypocotyls of COR-treated plants was the same in both genotypes (Fig. 4C).

Lower sensitivity of the 7B-1 *mutant to high COR corresponds to a higher expression of* NPR1 *gene under blue light*

In order to better understand the role of each signalling pathway of the observed reduced susceptibility of the *7B-1* mutant to COR, the expression pattern of the *NPR1* (*NON-EXPRESSOR OF PATHOGENESIS-RELATED1*) gene involved in the SA-signalling pathway, and of the *COI1* (*CORONATINE-INSENSITIVE1*) gene involved in the JA-signalling pathway were investigated. The highly specific and sensitive method q-RT-PCR was used to quantify and compare the changes in gene transcript amount in plants developed in dark, blue light, and red light, and after COR treatment.

In the absence of COR, the *NPR1* gene in the etiolated *7B-1* hypocotyl was expressed to a markedly greater extent than in the hypocotyl of WT plants. Blue light reduced essential amounts of *NPR1* transcripts in both genotypes, while the amount of mRNA in the *7B-1* hypocotyl was still significantly higher than in WT plants. Red light did not have a significant effect on *NPR1* expression in WT, but reduced it distinctly in the *7B-1* hypocotyl (Fig. 5A). COR treatment significantly reduced the expression of *NPR1* in etiolated WT and *7B-1* plants, so that the amount of *NPR1* mRNA was similar in both genotypes. Under blue light, COR did not noticeably influence the amount of *NPR1* transcripts in WT, but inhibited expression of the gene in the *7B-1* mutant. However, the expression of *NPR1* under COR stress in blue light was still essentially higher in *7B-1* than in WT hypocotyls. In red light, COR did not significantly influence the expression of the *NPR1* gene in WT or in the *7B-1* mutant (Fig. 5A).

As SA- and JA-signalling pathways are known to be antagonistic, expression of the *COI1* gene involved in JA signalling was also investigated. In the dark and in the absence of COR, expression of *COI1* in WT and *7B-1* hypocotyls reached a similar extent, and blue and red light had no significant effect on the amount of *COI1* mRNA (Fig. 5B). When etiolated seedlings of both genotypes were exposed to COR, the expression of *COI1* significantly decreased to a similar extent in both genotypes. COR also essentially reduced the expression of the *COI1* gene in blue light- and red light-developed hypocotyls, while the light effect was similar in both genotypes (Fig. 5B).

Discussion

In natural conditions, plants are continuously exposed to various abiotic and biotic stresses. In order to survive in such an environment, plants evolved sophisticated mechanisms that allow them to perceive external signals and to induce specific resistance responses. In crop species, abiotic and biotic stresses represent an important problem for breeders as they cause the loss of yield. Male-sterile mutants, spontaneous or induced, with reduced sensitivity to abiotic and biotic stress can be useful for crop producers

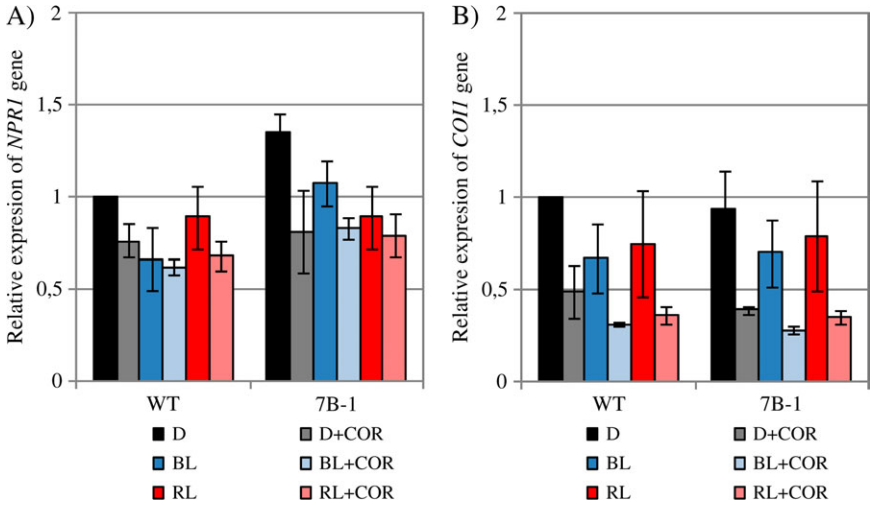

Fig. 5. q-RT-PCR quantification of coronatine (COR)-induced gene expression in hypocotyls from 5-d-old WT and *7B-1* tomato seedlings grown in dark (D), blue light (BL), and red light (RL): (A) *NPR1* gene expression; (B) *COI1* gene expression. Results are expressed relative to gene transcript amounts in WT plants developed in the dark and in the absence of COR. Results presented are averages ±SE obtained from two independent experiments.

and breeders (Fellner and Sawhney, 2001). Until now, in tomato, the only male-sterile *7B-1* mutant has been described showing a blue light-specific resistance to abiotic stress, for example, to high salinity and osmotic stress (Fellner and Sawhney, 2001, 2002). Using *in vivo* and *in vitro* assays, the present study demonstrated that seedlings of the *7B-1* mutant are less sensitive than WT to COR, the phytotoxine produced by several *P. syringae* strains. Similarly, in the case of resistance to abiotic stress, the significantly reduced responsiveness of *7B-1* to COR was observed only under blue light.

Effect of COR on the 7B-1 *mutant*

Physiological experiments in the greenhouse were conducted to determine the response of the *7B-1* mutant to COR. In no-stress plants, the *7B-1* mutant contains higher amounts of chlorophyll than the WT, confirming previous results (Fellner *et al.*, 2005; Sheoran *et al.*, 2006). During the time course of the experiments, formation of chlorotic areas by COR was observed in both genotypes, while COR was less efficient in inducing chlorosis expansion in the *7B-1* mutant. Concomitantly, chlorophyll degradation associated with chlorosis was less pronounced in the *7B-1* than in the WT. Chlorosis is the first clearly visible COR-induced symptom (Gnanamanickam *et al.*, 1982; Kenyon and Turner, 1990; Palmer and Bender, 1995; Tsuchiya *et al.*, 1999). Tsuchia *et al.* (1999) demonstrated that COR, as well as MeJA, induces an accumulation of chlorophyllase mRNA, the first key enzyme involved in chlorophyll degradation, catalysing the hydrolysis of the ester bond to yield chlorophyllide and phytol. In tobacco, the chlorosis correlated with the loss of chlorophyll *a* and *b* (Kenyon and Turner, 1990). Despite chlorosis, COR induces changes in the amino acid content with an increase in tryptophan and asparagine, and a drastic decrease in alanine and aspartate (Kenyon and Turner, 1990). Taken together, these results indicate that, even if in the final step *Pst* induces plant cell death (Nomura *et al.*, 2005), it remobilizes plant metabolism on its own during the initial phase of infection and development. Therefore, it is possible that in the *7B-1* mutant COR is less efficient in inducing chlorophyllase mRNA accumulation, leading to reduced chlorosis development.

In vitro assays were developed in order to investigate further the effect of COR-treatment on the development of tomato seedlings. As reported for different species (Weiler *et al.*, 1994; Bender *et al.*, 1999; Zhao *et al.*, 2003; Uppalapati *et al.*, 2005), COR induces important modifications of tomato seedling morphology: inhibition of root and hypocotyl growth, accumulation of anthocyanins, and chlorosis. In the present study, the extent of the change was COR dose-dependent in both genotypes, while the *7B-1* mutant exhibited a blue light-specific reduced sensitivity to COR. Fellner and Sawhney (2001), studying the response of the *7B-1* mutant to abiotic stress, demonstrated a close relationship between blue light and resistance to abiotic stress. Previous studies led to the hypothesis that the *7B-1* mutant could be defective in blue light perception and/or in the blue light-signalling pathway (Fellner and Sawhney, 2002). In this study, the results point to the fact that reduced responsiveness to blue light of the *7B-1* mutant is associated not only with resistance to abiotic, but also to COR-induced stress, suggesting that blue light can be an important factor involved not only in plant development but also in interaction with a biotic environment.

Unstressed *7B-1* plants contained less anthocyanin than unstressed WT plants, in agreement with data obtained by Sheoran *et al.* (2006). Under COR-stress, anthocyanin accumulation in seedlings was remarkable. The *7B-1* mutant accumulated substantially fewer anthocyanins than the WT, specifically in blue light. Anthocyanin synthesis is regulated by light and also by abiotic and biotic stress. In sorghum, inoculation of mesocotyls with the non-pathogenic fungus *Cochliobolus heterostrophus* strongly repressed expression of several genes involved in anthocyanin biosynthesis and induced synthesis of four phytoalexins (Lo and Nicholson, 1998). As anthocyanins and phytoalexins are synthesized through the same phenylpropanoid pathway, it was postulated that, under pathogen attack, plants were able to inhibit production of non-primordial compounds (anthocyanins) to favour production of compounds involved in the pathogen response. It is tempting to hypothesize that the reduced accumulation of anthocyanins in *7B-1* hypocotyls could be linked to increased accumulations of phytoalexins.

Role of hormones in plant growth responses to COR

It is well established that SA and JA are key factors in the resistance to biotic stress. Although the relationship between both signalling pathways has been over-simplified, it is accepted that SA- and JA-signalling pathways are mutually antagonistic (Anderson *et al.*, 2007). During the interaction between plants and *P. syringae*, the resistance is triggered by an SA-signalling pathway. In the present study, SA and JA were extracted and quantified from *7B-1* and WT hypocotyls grown under various light conditions, and in the absence or in the presence of COR. Concomitantly, expression of *NPR1* and *COI1* genes, involved in SA- and JA-signalling pathways, respectively, was studied as a function of light and COR concentration.

In untreated plants, the concentration of SA in mutant hypocotyls grown in dark and blue light was essentially reduced compared with that in WT plants. The reduced concentration of SA in the *7B-1* mutant correlated with essentially a greater amount of *NPR1* transcripts in the dark- and blue light-grown *7B-1* mutant. It was reported by others that, in *Arabidopsis*, SA induces expression of *NPR1* and consequently a pathogen response gene, such as *PR1*, in cooperation with a signalling pathway controlled by PhyA and PhyB photoreceptors (Devoto and Turner, 2005). The present data showed that, in *7B-1* but not in WT, red light reduces the expression of the *NPR1* gene relative to its expression in the dark. It suggests that, in red light, the functional product of the *7B-1* gene could be positively involved in an SA-signalling pathway. However, the

amount of the *NPR1* transcripts and the endogenous SA in red light-grown seedlings was similar in WT and the *7B-1* mutant, and it correlated with similar responses of both genotypes to the inhibitory effect of COR. In tomato hypocotyls, blue light negatively affects *NPR1* expression, but stimulates the accumulation of SA. It correlated with blue light-specific responsiveness to COR. This suggests that SA- and blue light-signalling pathways interact with each other, leading to the hypothesis that, in tomato, accumulation of SA may trigger the down-regulation of the SA-signalling pathway.

In response to COR, both genotypes accumulated SA and JA in hypocotyls, indicating that both pathways are likely to be involved in the susceptibility/resistance to COR in tomato. Concomitant accumulation of SA and JA during antimicrobial defence is not specific to tomato, since it has also been described in *Arabidopsis*, for instance (Block *et al.*, 2005; Laurie-Berry *et al.*, 2006). Despite an accumulation of defence hormones in both genotypes after a COR treatment, accumulation of SA was significantly greater in *7B-1* than in WT seedlings grown in blue light. It was associated with blue light-induced overexpression of the *NPR1* gene and reduced growth inhibition of the *7B-1* hypocotyl by COR under blue light. Conversely, a similar amount of *NPR1* transcripts in WT and *7B-1* hypocotyls treated by COR in red light correlated with similar hypocotyl growth responses to COR in both genotypes. A much greater accumulation of endogenous SA was observed in COR-treated *7B-1* hypocotyls than in WT when grown in the dark. This was associated with a slightly lower sensitivity of the etiolated mutant hypocotyl to the inhibitory effect of COR, but did not correlate with the amount of the *NPR1* transcripts, which was similar to that observed in etiolated COR-treated WT plants. This suggests that the blue light-signalling pathway including the *7B-1* gene plays a specific role in tomato responsiveness to COR.

The *COI1* gene encodes an F-box protein involved in the de-repression of the JA-signalling pathway through ubiquitination. The role of COR/MeJA in the JA-signalling pathway is well documented (Zhao *et al.*, 2003; Abramovitch and Martin, 2004; Laurie-Berry *et al.*, 2006; Katsir *et al.*, 2008). The quantitative PCR analysis of the expression of the *COI1* gene revealed that treatment of tomato plants with COR led to a down-regulation of the JA-signalling pathway. However, unlike differential expression of the *NPR1* gene in WT and the *7B-1* mutant, changes in the expression of the *COI1* gene were similar in both genotypes, and in all light conditions tested.

Resistance to *Pst* involves complex relationships between SA- and JA-signalling pathways, subjected to regulation by phytohormones, like ABA and auxin whose role in resistance to pathogens is well documented (Robert-Seilaniantz *et al.*, 2007; Wang *et al.*, 2007). Whereas the role of ABA in resistance to abiotic stress, seed germination, and plant growth is well known (Seo and Koshiba, 2002; Nambara and Marion-Poll, 2005), its role in resistance to pathogens is still poorly understood; several studies have shown negative as well as positive effects (reviewed in Mauch-Mani and Mauch, 2005). In tomato, ABA partially represses PAL activity upon infection with *Botrytis cinerea* (Audenaert *et al.*, 2002). Furthermore, Mohr and Cahill (2007) determined that exogenous ABA impairs the resistance of *Arabidopsis* to an avirulent *P. syringae* strain. This decreased resistance is associated with the reduction in lignin and SA accumulation. In soybean, exogenous ABA suppresses PAL activity and *PAL* mRNA accumulation in an incompatible reaction with *Phytophtora megasperma* f.sp. *glycinea* (Ward *et al.*, 1989). It is also possible that ABA can directly regulate the expression of some genes, like *PR1a* (*PATHOGENESIS-RELATED1*) whose promoter sequence contains a negative-acting ABA-responsive element TAACAAA (Audenaert *et al.*, 2002). Recently, de Torres-Zabala *et al.* (2007), using micro-array experiments, demonstrated that *Pst* hijacks the ABA-signalling pathway to cause disease in *Arabidopsis*. Here, a significant accumulation of ABA was observed in the hypocotyl of both genotypes treated with COR, with *7B-1* hypocotyls accumulating significantly higher concentrations of ABA, specifically in blue light. Despite the ABA overproduction, the

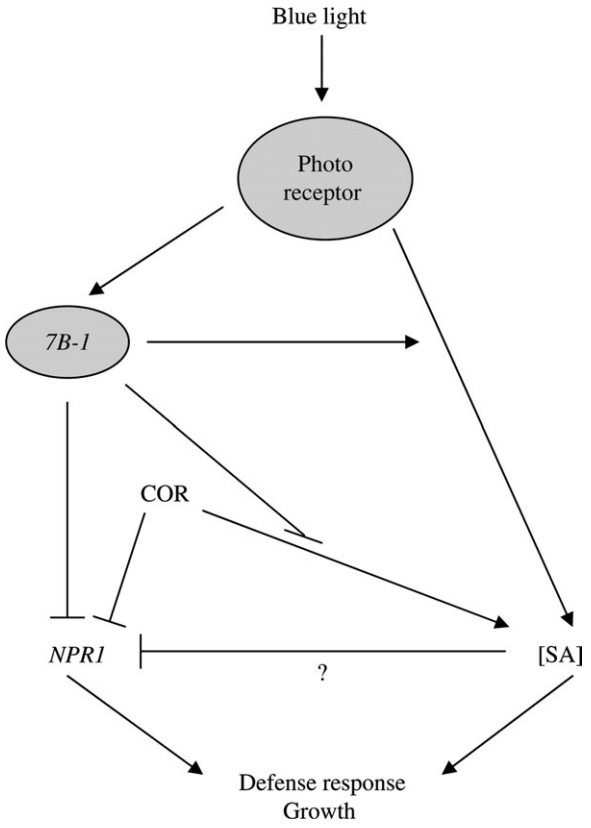

Fig. 6. Proposed schematic model illustrating the role of the *7B-1* gene in regulation of blue light- and coronatine (COR)-signalling pathways involved in the control of *NPR1* gene expression and the concentration of SA in tomato hypocotyl. Both blue light and COR increase the levels of endogenous SA in tomato hypocotyls. Conversely blue light and COR reduce levels of *NPR1* transcripts. Mutation in the *7B-1* gene reduces the blue light-signalling pathway, which results in greater expression of the *NPR1* gene, but a reduced concentration of endogenous SA.

7B-1 mutant showed reduced severity of COR-induced symptoms and accumulated higher amounts of *PAL* transcripts than WT (data not shown). Fellner *et al.* (2001) hypothesized that the *7B-1* mutant could be less sensitive to ABA than WT due to a default in ABA perception.

In summary, it became obvious that the *7B-1* mutant is less sensitive to COR-induced stress. Similarly, as reported for resistance of *7B-1* to abiotic stress (Fellner and Sawhney, 2001, 2002), significantly less responsiveness of the *7B-1* mutant to COR was observed only under blue light. In etiolated plants, the *7B-1* mutation results in an essentially lower concentration of endogenous SA, in elevated amounts of *NPR1* transcripts, and in a strong increase of SA concentrations by COR. Both blue light and COR increase the concentration of endogenous SA in tomato hypocotyls but, conversely, both blue light and COR reduce the expression of the *NPR1* gene in both genotypes. Since the *7B-1* mutant shows reduced blue light-specific sensitivity to COR, it is suggested that a functional product of the *7B-1* gene may play a role in both blue light- and SA-signalling pathways. A model is proposed in which the functional *7B-1* operates as a negative regulator of the blue light-controlled expression of *NPR1*, but also as a positive regulator of the blue light-mediated induction of SA in tomato hypocotyls. In addition, it is assumed that *7B-1* negatively regulates COR-induced accumulation of SA (Fig. 6). This model may explain the results of SA accumulation and *NPR1* expression.

Supplementary data

Figure S1: Effect on COR treatment (100 nM) on hormone content in hypocotyls of WT and *7B-1* tomato seedlings grown for 7 d in dark, blue light, and red light. (A, D) ABA concentration; (B, E) SA concentration; (C, F) JA concentration. A, B and C: data obtained during the second experiment; D, E and F: data obtained during the third independent experiment.

Acknowledgements

We thank Vipen K. Sawhney (University of Saskatchewan, Saskatoon, Canada) for providing seeds of the *7B-1* mutant. We also thank Pamela Lewis for her help in revising the English text. This research was supported by a grant from the Ministry of Education of the Czech Republic (grant no. MSM6198959215).

References

Abramoff M, Magelhaes P, Ram S. 2004. Image processing with ImageJ. *Biophotonics International* **11,** 36–42.

Abramovitch RB, Martin GB. 2004. Strategies used by bacterial pathogens to suppress plant defenses. *Current Opinion in Plant Biology* **7,** 356–364.

Anderson JP, Badruzsaufari E, Schenk PM, Manners JM, Desmond OJ, Ehlert C, Maclean DJ, Ebert PR, Kazan K. 2007. Antagonistic interaction between abscissic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *The Plant Cell* **16,** 3460–3479.

Audenaert K, De Meyer GB, Höffe MM. 2002. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. *Plant Physiology* **128,** 491–501.

Bender CL, Alarcon-Chaidez F, Gross DC. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiology and Molecular Biology Reviews* **63,** 266–292.

Block A, Schmelz EA, Jones JB, Klee HJ. 2005. Coronatine and salicylic acid: the battle between Arabidopsis and *Pseudomonas* for phytohormone control. *Molecular Plant Pathology* **6,** 79–83.

de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Egea PR, Bögre L, Grant M. 2007. *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *EMBO Journal* **26,** 1434–1443.

Devoto A, Turner JG. 2005. Jasmonate-regulated Arabidopsis stress signalling network. *Physiologia Plantarum* **123,** 161–172.

Fellner M, Franklin JA, Reid DM, Sawhney VK. 2005. Increased sensitivity to, and reduced production of, ethylene in an ABA-overproducing tomato mutant. *Acta Biologica Cracoviensia, Series Botanica* **47,** 205–212.

Fellner M, Sawhney VK. 2001. Seed germination in a tomato male-sterile mutant is resistant to osmotic, salt and low-temperature stress. *Theoritical and Applied Genetics* **102,** 215–221.

Fellner M, Sawhney VK. 2002. The *7B-1* mutant in tomato shows blue-light-specific resistance to osmotic stress and abscisic acid. *Planta* **214,** 675–682.

Fellner M, Zhang R, Pharis RP, Sawhney VK. 2001. Reduced de-etiolation of hypocotyl growth in a tomato mutant is associated with hypersensitivity to, and high endogenous levels of, abscisic acid. *Journal of Experimental Botany* **52,** 725–738.

Feys BJF, Benedetti CE, Penfold CN, Turner JG. 1994. Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *The Plant Cell* **6,** 751–759.

Gnanamanickam SS, Starratt AN, Ward EWB. 1982. Coronatine production *in vitro* and *in vivo* and its relation to symptom development in bacterial blight of soybean. *Canadian Journal of Botany* **60,** 645–650.

Hlaváčková V, Krchňák P, Nauš J, Novák O, Špundová M, Strnad M. 2006. Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. *Planta* **225,** 235–244.

Inskeep WP, Bloom PR. 1985. Extinction coefficients of chlorophyll *a* and *b* in *N*,*N*-dimethylformamide and 80% acetone. *Plant Physiology* **77,** 483–485.

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA. 2008. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proceedings of the National Academy of Sciences, USA* **105,** 7100–7105.

Kaul MLH. 1988. *Male sterility in higher plants*. Berlin: Springer Verlag.

Kenyon J, Turner JG. 1990. Physiological changes in *Nicotiana tabacum* leaves during development of chlorosis caused by coronatine. *Physiological and Molecular Plant Pathology* **37,** 463–477.

Krumm T, Bandemer K, Boland W. 1995. Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine- and isoleucince conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. *FEBS Letters* **377,** 523–529.

Kunkel BN, Brooks DM. 2002. Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology* **5,** 325–331.

Laurie-Berry N, Joardar V, Street IH, Kunkel BN. 2006. The *Arabidopsis thaliana JASMONATE INSENSITIVE 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **19,** 789–800.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell* **16,** 126–143.

Lo S-CC, Nicholson RL. 1998. Reduction of light-induced anthocyanin accumulation in inoculated sorghum mesocotyls. *Plant Physiology* **116,** 979–989.

Marten H, Hedrich R, Roelfsema MRG. 2007. Blue light inhibits guard cell plasma membrane anion channels in a phototropin-dependent manner. *The Plant Journal* **50,** 29–39.

Mauch-Mani B, Mauch F. 2005. The role of abscisic acid in plant-pathogen interactions. *Current Opinion in Plant Biology* **8,** 409–414.

Mitchell RE. 1985. Coronatine biosynthesis: incorporation of L-[U-^{14}C]isoleucine and L-[U-^{14}C]threonine into the 1-amino-1-carboxy-2-ethylcyclopropyl moiety. *Phytochemistry* **24,** 247–249.

Mohr PG, Cahill DM. 2007. Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in *Arabidopsis* infected with *Pseudomonas syringae* pv. *tomato*. *Functional Integrative Genomics* **7,** 181–191.

Murashige T, Skoog A. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiologia Plantarum* **15,** 473–497.

Nambara E, Marion-Poll A. 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56,** 165–185.

Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of Pathogenesis-Related (PR) protein genes in wounded mature tobacco leaves. *Plant and Cell Physiology* **39,** 500–507.

Nomura K, Melotto M, He SY. 2005. Suppression of host defense in compatible plant-*Pseudomonas syringae* interactions. *Current Opinion in Plant Biology* **8,** 361–368.

O'Donnell P, Schmelz EA, Moussatche P, Lund ST, Jones JB, Klee HJ. 2003. Susceptible to intolerance – a range of hormonal actions in susceptible *Arabidopsis* pathogen response. *The Plant Journal* **33,** 245–257.

Palmer DA, Bender CL. 1995. Ultrastructure of tomato leaf tissue treated with the pseudomonad phytotoxin coronatine and comparison with methyl jasmonate. *Molecular Plant-Microbe Interactions* **8,** 683–692.

Parry RJ, Mhaskar SV, Lin M-T, Walker AE, Mafoti R. 1994. Investigations of the biosynthesis of the phytotoxin coronatine. *Canadian Journal of Chemistry* **72,** 86–99.

Rangaswamy V, Jiralerspong S, Parry RJ, Bender CL. 1998. Biosynthesis of the *Pseudomonas* polyketide coronafacic acid requires monofunctional and multifunctional polyketide synthase proteins. *Proceedings of the National Academy of Sciences, USA* **95,** 15469–15474.

Rao MK, Uma Devi K, Arundhati A. 1990. Applications of genic male sterility in plant breeding. *Plant Breeding* **105,** 1–25.

Robert-Seilaniantz A, Navarro L, Bari R, Jones JD. 2007. Pathological hormone imbalances. *Current Opinion in Plant Biology* **10,** 372–379.

Santner A, Estelle M. 2007. The JAZ proteins link jasmonate perception with transcriptional changes. *The Plant Cell* **19,** 3839–3842.

Sawhney VK. 1997. Genetic male sterility. In: Shivanna K, Sawhney V, eds. *Pollen biotechnology for crop production and improvement*. Cambridge: Cambridge University Press, 183–198.

Sawhney VK. 2004. Photoperiod-sensitive male-sterile mutant in tomato and its potential use in hybrid seed production. *Journal of Horticultural Science and Biotechnology* **79,** 138–141.

Schmelz EA, Engelberth J, Alborn HT, O'Donnell P, Sammons M, Toshima H, Tumlinson JH. 2003. Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. *Proceedings of the National Academy of Sciences, USA* **100**, 10552–10557.

Seo M, Koshiba T. 2002. Complex regulation of ABA biosynthesis in plants. *Trends in Plant Science* **7,** 41–48.

Sheoran IS, Dumonceaux T, Datla R, Sawhney VK. 2006. Anthocyanin accumulation in the hypocotyl of an ABA-over producing male-sterile tomato (*Lycopersicon esculentum*) mutant. *Physiologia Plantarum* **127,** 681–689.

Shimazaki K, Doi M, Assmann SM, Kinoshita T. 2007. Light regulation of stomatal movement. *Annual Review of Plant Biology* **58,** 219–247.

Spoel SH, Koornneef A, Claessens SMC, *et al*. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. *The Plant Cell* **15,** 760–770.

Staswick PE, Tiryaki I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *The Plant Cell* **16,** 2117–2127.

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J. 2007. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature* **448,** 661–665.

Tsuchiya T, Ohta H, Okawa K, Iwamatsu A, Shimada H, Masuda T, Takamiya K-I. 1999. Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: finding of a lipase motif and the induction by methyl jasmonate. *Proceedings of the National Academy of Sciences, USA* **96**, 15362–15367.

Uppalapati SR, Ayoubi P, Weng H, Palmer DA, Mitchell RE, Jones W, Bender CL. 2005. The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. *The Plant Journal* **42,** 201–217.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* **3,** 1–12.

Wang D, Pajerowska-Mukhtar K, Culler HA, Dong X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology* **17,** 1784–1790.

Ward EWB, Cahill DM, Bhattacharyya MK. 1989. Abscisic acid suppression of phenylalanine ammonia-lyase activity and mRNA, and resistance of soybeans to *Phytophthora megasperma* f.sp. *glycinea*. *Plant Physiology* **91,** 23–27.

Weiler EW, Kutchan TM, Gorba T, Brodschelm W, Niesel U, Bublitz F. 1994. The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. *FEBS Letters* **345,** 9–13.

Xie D-X, Feys BJF, James S, Nieto-Rostro M, Turner JG. 1998. *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* **280,** 1091–1094.

Zhao Y, Thilmony R, Bender CL, Schaller A, He SY, Howe GA. 2003. Virulence systems of *Pseudomonas syringae* pv. *tomato* promote bacterial speck disease in tomato by targeting the jasmonate signaling pathway. *The Plant Journal* **36,** 485–499.