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ORIGINAL ARTICLE

Changes in plasma membrane aquaporin gene expression under osmotic stress and blue light in tomato

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Abstract

Divergent abiotic stresses induce osmotic stress on plant cells resulting in an imbalance in water homeostasis which is preserved by aquaporins. Since the plasma membrane aquaporins (PIPs) were shown to be involved in seed development and responses to abiotic stresses, we focused on determining the contribution of mannitol-induced osmotic stress, blue light (BL), and *7B-1* mutation to their gene expression in tomato (*Solanum lycopersicum* L.) seeds. To assess that, we used a quantitative RT-PCR to determine the expression profiles of genes encoding PIPs. Subsequently, a multiple linear regression analysis was used to evaluate the impact of studied stressors (mannitol and BL) and *7B-1* mutation on *PIP* gene expressions. We found that mannitol-induced osmotic stress and *7B-1* mutation (conferring the lower responsiveness to osmotic stress- and BL-induced inhibition of seed germination) decreased expression of *PIP1;3*, *PIP2;3* and *PIP1;2*, *PIP2;1* genes, respectively. This might be a way to retain water for radicle elongation and seed germination under the stress conditions. Interestingly, the expression of *PIP1;3* gene was downregulated not only by osmotic stress, but also by BL. Altogether, our data indicate the existence of a link between osmotic stress and BL signalling and the involvement of the *7B-1* mutation in this crosstalk.

Keywords Tomato · Seed · Aquaporins · Blue light · *7B-1* mutant · Mannitol · *PIP*s

Introduction

Plant growth and development are often severely affected by abiotic stresses. Abiotic stresses are known to induce osmotic imbalance in plants and disturb the plant water homeostasis. Aquaporins, the membrane intrinsic proteins facilitating and regulating the passive movement of water molecules down a water potential gradient, play a central role in maintaining a turgor and water transport in plants (Maurel et al. 2015). Above all, the plasma membrane intrinsic protein (PIP) aquaporins have been proven to participate in responses to abiotic stresses, such as drought, salinity, or chilling (Lian et al. 2004; Liu et al. 2007). Moreover, *PIP* gene expression can be regulated by these abiotic stresses (Aroca et al. 2012) as well. In addition, there is an opinion that PIP1-type aquaporins can function as sensors of blue light (BL) because of their localization in the plasma membrane and ability to bind flavins (the cofactors of BL photoreceptors) (Kaldenhoff and Eckert 1999; Lorenz et al. 2003). Furthermore, the expression of *PIP1;2* aquaporin was showed to be enhanced by BL in *Arabidopsis thaliana* cell tissue cultures (Kaldenhoff et al. 1995, 1996).

In plant seeds, aquaporins play a central role in the physiology of water economy (Maurel et al. 2015; Obroucheva 2013; Obroucheva et al. 2017). Embryo growth during seed germination is driven by water uptake which is necessary for embryo cell elongation and expansion. In general, seed germination follows three phases. In phase I, imbibition of water, reactivation of metabolism and at the end of this phase, cell elongation, and radicle emergence occurs. In phases II and III, the embryonic axis grows further as cells elongate and divide to establish the seedling (Toole et al. 1956). Aquaporins are not involved in the early imbibition of dry seed by water, but they play a role in embryo growth (Obroucheva 2012; Willigen et al. 2006). It was reported that

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the expression of aquaporin genes is low during seed imbibition and starts when a seed hydration level of 50–55% (fresh weight) is achieved (Obroucheva 2012). The expression of aquaporin genes is strongly activated at radicle emergence and just after it. Despite dry seeds contain the transcripts of aquaporin genes and aquaporin themselves, it seems that the aquaporins function only after radicle protrusion to provide enhanced water inflow to growing cells (Obroucheva 2013). In addition, Shiota et al. (2006) showed that plasma membrane aquaporins are expressed during tomato seed development. Apparently, they participate in the delivery of water and possibly some other compounds into the developing seeds (Obroucheva 2013).

Seed germination is one of the physiological processes which are influenced by light. Although very little is known about perception of blue light (BL) by seeds (Goggin and Steadman 2012), it was shown that BL mostly reduces seed germination in tomato and that tomato seeds under osmotic stress germinate better in the dark than in BL (Fellner and Sawhney 2002; Piterková et al. 2012). Besides, BL can modulate transcription of many genes including plant aquaporins (Baaziz et al. 2012; Kaldenhoff et al. 1995, 1996) and water stress acts on aquaporin function at the level of transcription and gating through direct effects on osmotic gradient (Luu and Maurel 2005).

Tomato (*Solanum lycopersicum* L.) is one of the most important crop species. The *7B-1* mutant displays male sterility under long days, but under short days, it produces fertile flowers (Sawhney 1997; Sheoran et al. 2009). Compared with the corresponding WT (cv. Rutgers), the *7B-1* shows reduced de-etiolation of hypocotyl growth associated with hypersensitivity to, and high endogenous levels of, ABA (Fellner et al. 2001). The *7B-1* mutation impairs various BL responses, such as BL-induced stomata opening (Hlavinka et al. 2013; Ježilová et al. 2012), hypocotyl phototropism (Bergougnoux and Fellner, unpublished results) and chloroplast movement (Špundová and Savara, unpublished results). It suggests that *7B-1* mutation may somehow affect phototropin signalling, while the possibility that *7B-1* is a CRY mutant is not likely (Sheoran et al. 2006). In long-day conditions, *7B-1* seed germination was found to be less responsive than corresponding WT to various salts (including NaCl, Na_2SO_4, KCl, and K_2SO_4), osmotic stress and to low-temperature stress (Fellner and Sawhney 2001). Interestingly, Fellner and Sawhney (2002) also reported that seed germination in *7B-1* is more tolerant to exogenous ABA and osmotic stress specifically under BL. Moreover, we reported that BL amplifies the inhibitory effect of osmotic stress on tomato seed germination (Fellner and Sawhney 2002; Piterková et al. 2012). Recently, we identified number of potentially novel miRNAs, which are associated with enhanced tolerance of *7B-1* to abiotic stress under BL (Omidvar et al. 2015). Our data also showed that response to different lights and stresses in *7B-1* and WT involves remodelling DNA methylation, highlighting the differences in epigenetic and transcriptional regulation of light and stress responses between *7B-1* and WT (Omidvar and Fellner 2015). Specifically in BL conditions, *7B-1* is less sensitive to biotic stress as well (Bergougnoux et al. 2009). Recently, a genetic characterization of the *7B-1* mutant was published and *SlGLO2* gene was proposed as a candidate gene underlying the *7B-1* mutation (Pucci et al. 2017). *SlGLO2* belongs to class B MADS-box genes which are involved in stamen development (Pucci et al. 2017). However, a role of *SlGLO2* in BL- and/or stress signalling is not known and it is under investigation.

Number of reports describing a direct contribution of BL in the ability of plants to tolerate abiotic stress is very limited. Therefore, *7B-1* mutant seems to be a suitable model to study a possible interaction between BL and osmotic stress. Various environmental factors regulate aquaporins mostly at the transcriptional level (Liu et al. 2013). For this reason, we hypothesized that the tolerance of *7B-1* mutant seeds to BL and osmotic stress may involve differential expression of aquaporin genes. Shiota et al. (2006) showed that genes of PIP aquaporin family (namely *PIP1;2*, *PIP1;3*, *PIP1;4*, *PIP1;5*, *PIP2;1*, *PIP2;2,* and *PIP2;3*) participate in tomato seeds development, while expression of some *PIPs* can be regulated by abiotic stresses (Aroca et al. 2012). Thus, we focused on this aquaporin family and we studied the expression of these seven genes in *7B-1* seeds as a function of mannitol-induced osmotic stress and BL conditions. We revealed that the gene expression patterns of *PIP1;2*, *PIP1;3*, *PIP2;1,* and *PIP2;3* were altered in response to BL, osmotic stress, and/or by *7B-1* mutation. Interestingly, both *7B-1* mutation and osmotic stress decreased expression of some of the studied *PIPs*, most probably to preserve enough water for radicle elongation under stress conditions. As *7B-1* mutation causes defects in BL responses, and expression of *PIP1;2* and *PIP2;1* is regulated by BL, we hypothesized that these genes may play roles in BL sensing or signalling.

Materials and methods

Plant material and stress treatment

Solanum lycopersicum L. (tomato) seeds of cultivar Rutgers (WT) and recessive single gene *7B-1* mutant (Sawhney 1997) were used. *7B-1* seeds are less responsive than WT to BL-induced inhibition of seed germination and to various abiotic stresses specifically under BL (Fellner and Sawhney 2001, 2002; Piterková et al. 2012).

All experiments were performed in in vitro conditions. Sterilized seeds (2.8% sodium hypochlorite solution, Bochemie, Czech Republic) were incubated in squared Petri dishes

filled with basal MS medium (Murashige and Skoog 1962) or with MS medium supplemented with 20 or 70 mM mannitol. The edge of each plate was twice sealed with an air permeable tape (Batist, Czech Republic). The dishes with seeds were placed in vertical position into controlled growth chambers (Microclima 1000E, Snijders Scientific B. V., The Netherlands) in continuous BL conditions or they were wrapped in tinfoil (to simulate the darkness) and placed into the same chamber. BL was provided by blue tubes TLD-36W/18-Blue (Philips, USA) with a maximum irradiance at 460 nm. Total photon fluence rate of light was 10 µmol m^{-2} s^{-1}. The light spectrum was measured using a portable spectroradiometer (model LI-1800; Li-COR, NE, USA).

Number of germinated seeds was counted the seventh day after sowing and the average percentage of seed germination was calculated. At least 11 experiments were done for each treatment. To show a trend in course of seed germination, the number of germinated seeds was scored from second to seventh day as well. A control on MS was sown for each experiment. For each treatment, there were at least four independent experiments per each time point.

Gene expression analyses

The WT and *7B-1* mutant seeds germinated on basal MS medium or MS medium supplemented with 70 mM mannitol were collected (96 h after sowing, radicle 1–10 mm long) into liquid nitrogen. Seeds from BL were harvested under BL and seeds from darkness under green safelight. Harvested seeds were ground under liquid nitrogen and total RNA was isolated with the Isolate II RNA Plant Kit (Bioline) using RLS buffer according to a manufacturer´s instruction. At the last step, RNA was eluted with 40 µl of RNase-free water. As soon as isolation was done, residual DNA was removed from samples by recombinant DNaseI treatment (Takara Bio Inc., Japan) and recombinant RNase inhibitor (Takara Bio Inc., Japan) for 60 min at 37 °C. DNaseI was inactivated by heat treatment according to manufacturer´s instruction. Following this, first-strand cDNA mixtures were prepared from 0.7 µg of total RNA using PrimeScript™ 1st strand cDNA Synthesis Kit (Takara Bio). The gene expression was analysed using SensiFAST™ SYBR Lo-ROX Kit (Bioline) on the CFX96 Touch™ (BioRad, USA). The PCR reaction mixture was prepared according to manufacturer's protocol—the reaction mixture contained 10 µl of SensiFast SYBR^R Lo-ROX Mix, 0.8 µl of each primer (10 µM), 4.4 µl of sterile RNase-free water, and 4 µl of cDNA template (diluted 1/50). The final reaction volume was 20 µl. Each sample was measured in triplicate. The following PCR running conditions were used—an initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 15 s. Melting curve was analysed at the end of PCR reaction. The experiments were repeated five times.

PP2Acs (protein phosphatase 2A catalytic subunit) (Lovdal and Lillo 2009) and *TIP41-like* (Dekkers et al. 2012) genes were used as reference genes. The sequences of *PIP* primers used are given in Table 1. The primers were designed using PrimerQuest® program (IDT, Coralville, USA). The primer efficiencies were calculated from the slope of the dilution curve and they ranged from 0.9 to 1.0.

Statistical analysis

The significant differences between control (seeds on basal MS medium) and mannitol treatments were tested using Kruskal–Wallis test (a distribution-independent test) with multiple comparisons at the 0.05 significance level, since the data were not normally distributed. The analysis was done separately for dark- and BL-grown samples. For each treatment, the significance of differences between the dark and BL was assessed using Mann–Whitney *U* test (a non-parametric test) at the 0.05 significance level. Statistical analyses were performed using the STATISTICA 12 software (StatSoft, OK, USA). The results of germination assay are presented as box plots, where the lower and upper ends of the box are the first and third quartiles, respectively. The horizontal line inside the box is the median value (the square inside the box represents a mean) and the whiskers indicate the adjacent values (1.5 times interquartile range).

The relative gene expression of target gene was determined against the expression level of both reference genes with taking into account the different PCR efficiencies of

Table 1 Sequences of *PIP* primers used in RT-qPCR analyses

Gene	Accession number	Forward primer	Reverse primer
Sl*PIP1;2*	BP884557	TCCTATTTTGGCACCTCTTCC	ATCCCATGCCTCGTCTTTG
Sl*PIP1;3*	AW625013	ACGAAAGGTGATGGTCTTGG	GGGAACGTGTGAATCTCTAGC
Sl*PIP1;4*	AF218774	GAAGAGGATGTGAAGGTTGGAG	ACCAAGAATGTAGCTCACCAG
Sl*PIP1;5*	X73848	CACCAGCTCCATTGTTTGAAC	AACCCCTTGAATACCCACTG
Sl*PIP2;1*	BI929127	AGGTGGAGGTGCTAACTTTG	AACACAGGGACATGGGAATC
Sl*PIP2;2*	BG128835	CAGCATGGCAAAGATTATGTGG	CGTAGAGGAAAAGCAGAGTAGC
Sl*PIP2;3*	AW224678	TTCACCTTGCCACTATTCCG	AGAAAATCCAGTGTTCGTCCC

genes (Pfaffl 2001). Then, a geomean of the calculated relative expressions was used as a dependent variable to design a matrix for multiple linear regression analysis. The independent variables (predictors) designed as a mutation, light, and stress could take on one of two values, 1 or 0, to indicate the presence or absence, respectively, of the effect of the predictor (the binary system). To find the significance of the effect of predictors and to quantify it, the multiple linear regression analysis was done using the QC.Expert 2.9 software (TriloByte Statistical Software, Czech Republic).

Results

The main goal of this work was to investigate how osmotic stress (induced by 70 mM mannitol) and BL influence the expression profile of *PIP* genes in *7B-1* mutant seeds and its corresponding WT and how their gene expression patterns associate with reduced *7B-1* sensitivity to osmotic stress and BL. We focused on the effect of two different mannitol treatments and BL on seed germination in both genotypes. Then, we studied changes in the expression of *PIP* genes in seeds induced by 70 mM mannitol, BL and *7B-1* mutation.

Seed germination assays

In the dark as well as in BL, WT seed germination was significantly affected by treatment with 70 mM mannitol, but not by 20 mM mannitol (Fig. 1a). Similarly, seed germination in *7B-1* mutant was reduced significantly by 70 mM mannitol in the dark as well as in BL (Fig. 1b). However, in the dark, 70 mM mannitol tended to reduce *7B-1* mutant seed germination less than WT seed germination, but this difference did not reach statistical significance (at the 0.05 significance level). In both the dark and BL, 20 mM mannitol had not significant effect on *7B-1* mutant seed germination (Fig. 1b).

For each treatment, the effect of BL on WT and *7B-1* mutant seed germination compared to D was assessed using Mann–Whitney test (at the 0.05 significance level). The effect of BL was always significant (not marked in Fig. 1).

The trends of kinetics of seed germination shown in Fig. 2 indicate that WT and *7B-1* mutant differed just very slightly in the kinetics of seed germination on the basal MS medium in the dark. On the other hand, BL reduced markedly kinetics of WT seed germination (Fig. 2a), whereas in *7B-1* mutant, its effect was not so pronounced (Fig. 2b).

Changes in aquaporin gene expression under blue light and osmotic stress

In this study, we focused on investigation into the effect of BL, osmotic stress induced by 70 mM mannitol and *7B-1* mutation on RNA levels of plasma membrane aquaporins (namely, *PIP1;2*, *PIP1;3*, *PIP1;4*, *PIP1;5*, *PIP2;1*, *PIP2;2,* and *PIP2;3*) in germinated seeds (radicle up to 10 mm) cultivated for 96 h.

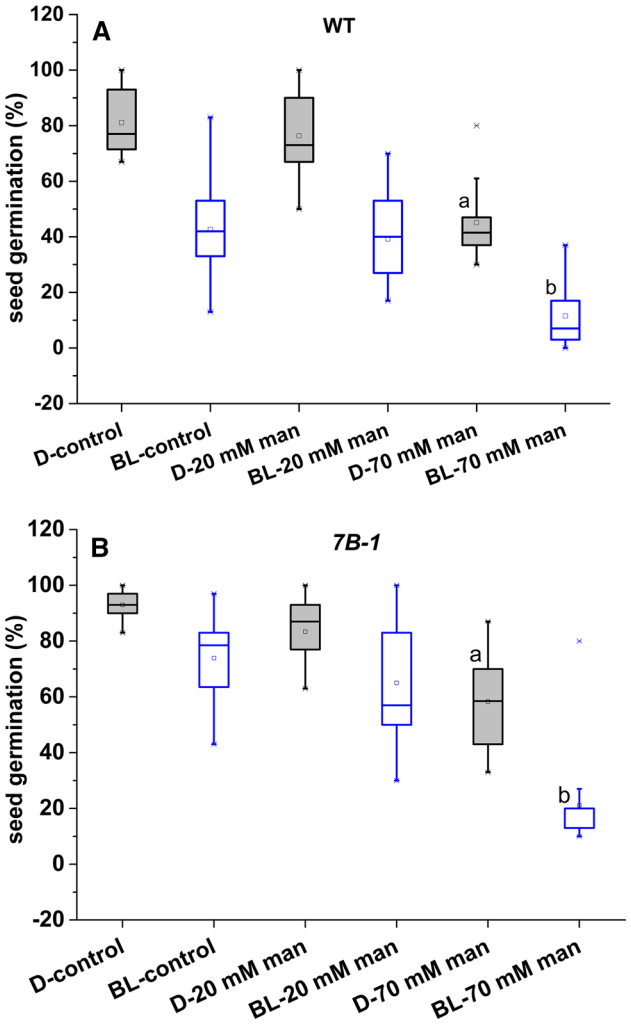

Fig. 1 Effect of mannitol on germination of tomato cv. Rutgers (WT) (**A**) and *7B-1* mutant (**B**) seeds incubated for 7 days in the dark (D) or under continuous blue light (BL). Seeds germinated on the basal MS medium (MS) serve as a control. The significant difference of mannitol treatments compared to corresponding control on medium without mannitol was tested using Kruskal–Wallis test at 0.05 significance level, a: in the dark, significantly different from D-control, b: in BL, significantly different from BL control. For each treatment, at least 11 experiments were done

The multiple linear regression analysis showed that none of studied predictors (blue light, osmotic stress, and *7B-1* mutation) reached statistical significance (at 0.05 level) for *PIP1;4*, *PIP1;5,* and *PIP2;2* genes (Table 2). In other words, expression of none of these genes was affected significantly by BL, osmotic stress, or by *7B-1* mutation. With the predictors mutation and stress held fixed, BL affected significantly the expression of *PIP1;2* (upregulation),

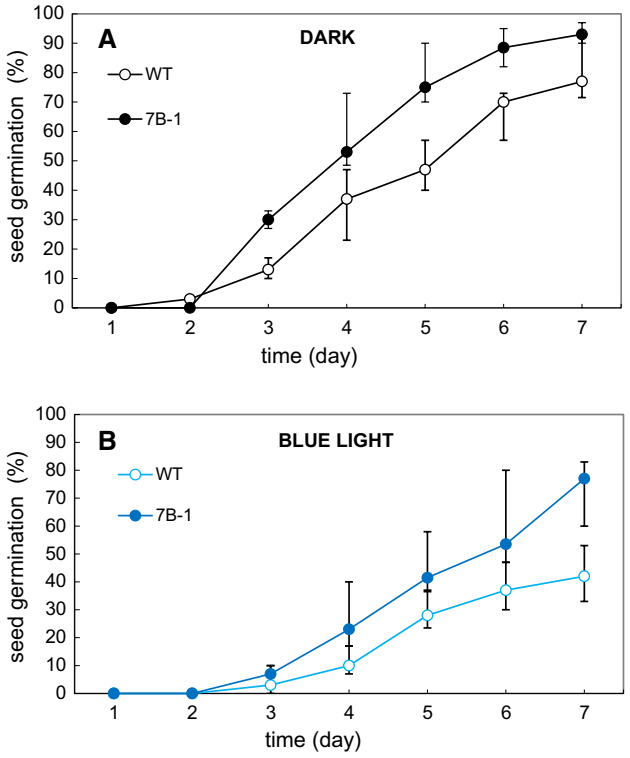

Fig. 2 Kinetics of WT and *7B-1* mutant seed germination on MS medium (control) and on MS medium supplemented with 70 mM mannitol during 7-day cultivation in the dark (**A**) or blue light (**B**). The data show medians and the error bars represent the first and third quartiles. For each treatment, there were at least four independent experiments per each timepoint. Thirty seeds per each dish were sown for each treatment

PIP1;3 (downregulation), and *PIP2;1* (downregulation) genes (Table 3). As Table 3 further shows, *7B-1* mutation decreased significantly the expressions of *PIP1;2* and *PIP2;1* genes when all the other predictor variables (BL and osmotic stress) were held fixed. Finally, held all the other predictors constant, the mannitol-induced osmotic stress reduced significantly the expression levels of *PIP1;3* and *PIP2;3* genes (Table 3).

In other words, the results of multiple linear regression analysis can also be presented as the equation of multiple linear regression involving the predictors with significant impact on the expression of each gene (Table 4).

Discussion

Expression of the aquaporins is divergently regulated by various environmental factors as a part of an adaptation mechanism by which plants reduce water losses through efflux to cope with stress conditions. Since the specific function of plant aquaporins under different stress and light conditions is still unclear, the comprehensive studies of aquaporin gene expression in divergent plant species are crucial to improve our understanding of their function in responses to various challenges. We wanted to assess statistically the potential impact of 70 mM mannitol-induced osmotic stress, BL and *7B-1* mutation on *PIPs* gene expression. The expression patterns could provide useful indications about the functions of the studied aquaporins. Tomato *7B-1* mutant seed germination, showing BL-specific reduced responsiveness to osmotic stress (Fellner and Sawhney 2002), is a valuable model system to study the possible involvement of aquaporins in light-regulated stress signalling.

Various responses of aquaporins to osmotic stress suggest that each aquaporin isoform could contribute differently to water transport and regulation of water homeostasis in germinated seeds. Several studies demonstrated that plants downregulate the aquaporin expressions to avoid excessive loss of water in challenging environment (Alexandersson et al. 2005; Jang et al. 2004; Lian et al. 2006). Here, we found that the expressions of *PIP1;3* and *PIP2;3* genes were downregulated by osmotic stress induced by 70 mM mannitol and it was associated with reduction of seed germination. All these literature data suggest that some aquaporins may help to adapt or tolerate the stress by reducing their expression. On the other hand, other aquaporins might help in maintaining the normal physiological processes in seeds, and thus, their expressions remained stable under the stress conditions. In our study, this was the case of *PIP1;2*, *PIP1;4*, *PIP1;5*, *PIP2;1,* and *PIP2;2* aquaporin genes. On the contrary, some reports show that the expression of *PIPs* can

Table 2 Results of a multiple linear regression analysis for genes *PIP1;4*, *PIP1;5*, and *PIP2;2*, whose gene expression was not affected significantly by blue light (BL), *7B-1* mutation, or mannitol-induced osmotic stress

Predictors	*PIP1;4*		*PIP1;5*		*PIP2;2*	
	Regression coefficient	*p* value	Regression coefficient	*p* value	Regression coefficient	*p* value
Constant	**1.57**	0.0000	**12.51**	0.0000	**1.04**	0.0000
BL	− 0.17	0.3859	0.75	0.3042	− 0.05	0.3949
Mutation	0.30	0.1224	− 0.28	0.7027	− 0.11	0.0666
Stress	0.06	0.7334	− 0.14	0.8433	− 0.06	0.2950

Significant values are shown in bold

In the table, the value of regression coefficients and corresponding *p* values are shown

Table 3 Results of a multiple linear regression analysis showing the effect of blue light (BL), *7B-1* mutation and mannitol-induced osmotic stress on the expression of *PIP* genes

Predictors	*PIP1;2*		*PIP2;1*		*PIP1;3*		*PIP2;3*	
	Regression coefficient	*p* value	Regression coefficient	*p* value	Regression coefficient	*p* value	Regression coefficient	*p* value
Constant	**8.35**	0.0000	**10.09**	0.0000	**0.63**	0.0000	**10.32**	0.0000
BL	**0.73**	0.0126	**− 0.52**	0.0321	**− 0.21**	0.0001	− 1.01	0.0723
Mutation	**− 0.59**	0.0417	**− 0.53**	0.0294	− 0.07	0.1302	− 0.57	0.3032
Stress	− 0.20	0.4734	0.07	0.7645	**− 0.10**	0.0435	**− 1.52**	0.0083

Significant values are shown in bold

In the table, the value of regression coefficients and corresponding *p* values are shown. The constant is the expression level of the gene when all of the studied predictors are equalled to zero

Table 4 Equations of a multiple linear regression for *PIP1;2*, *PIP1;3*, *PIP2;1,* and *PIP2;3* genes

Gene	Equation of multiple linear regression
PIP1;2	$R = 8.35 + 0.73$ (light) $- 0.59$ (mutation)
PIP1;3	$R = 0.63 - 0.21$ (light) $- 0.10$ (stress)
PIP2;1	$R = 10.09 - 0.52$ (light) $- 0.53$ (mutation)
PIP2;3	$R = 10.32 - 0.52$ (stress)

Shown are the values of regression coefficient of the predictors (light, mutation, or/and mannitol-induced osmotic stress) with significant (at the 0.05 significance level) impact on gene expression (*R*)

also be upregulated by abiotic stresses, suggesting that these aquaporins can facilitate water transport under stress conditions (Jang et al. 2004; Liang et al. 2013; Liu et al. 2013).

Although the difference in percentage of WT and *7B-1* mutant seed germination under 70 mM mannitol was not so distinct as reported for higher mannitol concentrations (Fellner and Sawhney 2002), *7B-1* mutation had a statistically significant negative effect on expression of aquaporin genes *PIP1;2* and *PIP2;1*. This indicates the tendency of *7B-1* mutant seeds to preserve water for radicle growth during seed germination. Thus, the mutation acted on *PIP* gene expressions in the similar way as osmotic stress (conserve the water under the stress) making *7B-1* mutant pre-adapted to osmotic stress. This is further supported by the elevated level of ABA in *7B-1* mutant (Fellner and Sawhney 2001, 2002).

Blue light tended to modulate *PIP* gene expression levels in WT and *7B-1* mutant seeds (Balarynová and Fellner, unpublished results). Here, we confirmed statistically this trend and found that BL enhanced *PIP1;2* and decreased *PIP2;1* and *PIP1;3* gene expressions in tomato seeds. It is known that BL reduces tomato seeds germination (radicle protrusion) (Piterková et al. 2012 and here Fig. 2). On the other hand, BL stimulates tomato root elongation (Balarynová and Fellner, unpublished results); thus, the same stimulatory effect could be expected for radicle. Therefore, the effect of BL on expression of *PIPs* genes involved in radicle elongation during seed germination could be dual. *PIPs* expression should be sufficient to ensure the radicle elongation but not so high to prevent immoderate loses of water which is important for cell elongation.

Literature reporting participation of aquaporins in seed germination or radicle growth is very limited (Obroucheva 2012; Obroucheva et al. 2017, Willigen et al. 2006). Interaction between light and aquaporins was studied especially in plant leaves because of their putative involvement in regulation of water transport and turgor in leaf cells. It was showed that plasma membrane aquaporins play a role in response of the leaf hydraulic conductance to light which stimulates leaf conductance to water. The light stimulation of leaf hydraulic conductance was not associated with stomatal opening. For example, *Juglans regia* aquaporin *JrPIP2;1* and *JrPIP2;2* transcript abundances are related to the dynamics of water transport in leaves (Cochard et al. 2007). Interestingly, BL and green light seem to stimulate leaf hydraulic conductance the most effectively (Baaziz et al. 2012). In our experiments, BL reduced significantly the percentage of seed germination and it was associated with downregulation of *PIP1;3* and *PIP2;1* genes. These results suggest that these PIPs might play an important role in water transport during seed germination. On the other hand, expression of *PIP1;2* gene was induced by BL. Aquaporin PIP1;2 seems to be important element of a whole plant water status in *A. thaliana*. Its expression and activity was reported to be control by multiple factors including BL (Kaldenhoff et al. 1993; Postaire et al. 2010). Besides, *PIP1;2* was one of the most transcribed aquaporin in germinated tomato seeds (Tables 3, 4). Thus, we can expect its significant contribution to maintain appropriate water status in tomato seeds under BL conditions. Moreover, *PIP1;2* may compensate for the reduced expression of *PIP1;3* and *PIP2;1* genes. Finally, *PIP1;2* gene could perform another roles in seeds (see below), because it is believed that PIP2 aquaporins are better water channels than PIP1 aquaporins (Maurel et al. 2015).

Aquaporins transport not only water but also other solutes of great physiological importance such as carbon dioxide, hydrogen peroxide or oxygen. For example, AtPIP1;2 seems to be a CO_2 transporter (Uehlein et al.

2003, 2012) and *Nicotiana tabacum* aquaporin PIP1;3 is thought to be involved in oxygen transport. Thus, PIPs appear to play a dual function. They are important for preserving water homeostasis and also participate in regulation of photosynthesis or cell signalling. AtPIP1;4 is able to translocate extracellular hydrogen peroxide into the cytoplasm and active immune responses induced by bacteria (Tian et al. 2016).

There is a hypothesis that PIP1 aquaporins function as sensors of BL, which is supported by their localization in the plasma membrane and some of their structural features (Kaldenhoff and Eckert 1999; Lorenz et al. 2003). Interestingly, in our experiments, we showed that expression of *PIP1;2* is significantly stimulated by BL but significantly reduced in *7B-1* mutant (Table 3). Then, the reduction of *PIP1;2* expression in the mutant is in agreement with the fact that *7B-1* mutation confers reduced plant and/or seed responsiveness to BL (Fellner et al. 2001; Fellner and Sawhney 2002; Hlavinka et al. 2013; Ježilová et al. 2012). Therefore, we agree that PIP aquaporins could be more than water transporters in seeds.

Altogether, we showed that both osmotic stress and *7B-1* mutation decreased the expression levels of four tested *PIP* genes, probably to protect seed against excessive water loss during the seed germination under the osmotic stress. Moreover, BL affected *PIP* transcription levels indicating the connection between BL signalling and aquaporins. Thus, the inhibitory effect of BL and osmotic stress on tomato seed germination could be, at least partly, related to changes in aquaporin transcripts abundance. As *7B-1* mutation causes defect in phototropin responses (Hlavinka et al. 2013; Bergougnoux and Fellner, unpublished data), we hypothesize that *PIP1;2* and *PIP2;1* may play a role in BL sensing or signalling. This is supported by the fact that the expression of both genes is regulated by BL. Other experiments based on analysis of tomato phototropin mutants would support or disprove this hypothesis.

Author contribution statement JB performed and designed all experiments and wrote the manuscript. JD made data analyses and edited the manuscript. MF designed the experiments and edited the manuscript.

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