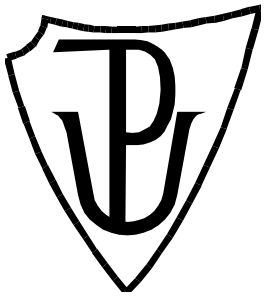
PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science

Laboratory of Growth Regulators



Interaction of blue light and brassinosteroid signalling in plant growth

MASTER'S THESIS

Author: **Bc. Lucie Pilařová**

Study Programme: N1501 Plant Physiology

Supervisor: **doc. RNDr. Martin Fellner, Ph.D.**

Year: 2016

Bibliographical identification

Author's first name and surname	Lucie Pilařová
Title of thesis	Interaction of blue light and brassinosteroid signalling in plant growth
Type of thesis	Master
Department	Laboratory of Growth Regulators, Group of molecular physiology
Supervisor	doc. RNDr. Martin Fellner, Ph.D.
The year of presentation	2016
Abstract	The presented Master's thesis focuses on the interaction between blue light and the brassinosteriod signalling pathway in the growth of tomato mutant *7B-1*. The subject of the thesis was based on a previous study where the altered responses of the *7B-1* mutant to an exogenous brassinosteroid were reported for the first time (Pilařová, 2014). The theoretical part of this work consists of a literature review summarising the research on the mutant *7B-1*, brassinosteroid and light signalling and their interactions. The experimental part presents the results obtained by the method RT-qPCR in a series of experiments which examined the expression of the brassinosteroid receptor *BRI1* in *7B-1* and wild-type tomato seedlings. The results suggest that the *BRI1* expression level is affected by blue light and that *7B-1* hypocotyls may express less *BRI1* compared to the wild-type.
Keywords	tomato, *7B-1*, brassinosteroids, *BRI1*, RT-qPCR 24-*epi*brassinolide, blue light, *BRI1* expression
Number of pages	44
Number of appendices	0
Language	English

Bibliografická identifikace

Jméno a příjmení autora	Lucie Pilařová
Název práce	Interakce signálních drah modrého světla a brassinosteroidů v růstu rostlin
Typ práce	Magisterská
Pracoviště	Laboratoř růstových regulátorů, Skupina molekulární fyziologie
Vedoucí práce	doc. RNDr. Martin Fellner, Ph.D.
Rok obhajoby práce	2016

Abstrakt

Předkládaná magisterská práce se věnuje interakci modrého světla a brasinosteroidní signální dráhy v růstu mutanta rajčete *7B-1*. Téma vychází z bakalářské práce, ve které byly poprvé popsány změny v reakcích mutanta *7B-1* k exogennímu brassinosteroidu (Pilařová, 2014). Teoretická část práce nabízí přehled odborné literatury, týkající se výzkumu mutanta *7B-1*, signální dráhy světla, brassinosteroidní signální dráhy a jejich interakce. Experimentální část představuje výsledky získané metodou RT-qPCR v řadě experimentů, které se zabývaly expresí brassinosteroidního receptoru *BRI1* v semenáčcích mutanta *7B-1* a standardního genotypu. Výsledky naznačují, že hladina exprese *BRI1* je ovlivněna modrým světlem, a že hypokotyly mutanta *7B-1* mohou exprimovat méně *BRI1* v porovnání se standardním genotypem.

Klíčová slova	rajče, *7B-1*, brassinosteroidy, *BRI1*, RT-qPCR 24-*epi*brassinolid, modré světlo, exprese *BRI1*
Počet stran	44
Počet příloh	0
Jazyk	Anglický

I hereby declare that the work presented in this Master's thesis is my own and it was carried out under the supervision of doc. RNDr. Martin Fellner, Ph.D. All the primary and secondary sources are listed in the References section.

In Olomouc, on

Acknowledgements

I would like to thank several people for their kind support. I am most grateful to my supervisor, doc. RNDr. Martin Fellner, Ph.D. for his invaluable guidance. I also would like to thank other members of the Group of Molecular Physiology, especially Mgr. Jana Balarynová for discussing the experiments with me. Finally, I want to thank K. B. for his great help, for being mad as a March hare and making me laugh.

Contents

1 INTRODUCTION

The presented Master's thesis focuses on the interaction between blue light and the brassinosteriod signalling pathway in growth of the tomato mutant *7B-1* which was isolated for its male-sterility depending on the photoperiod (Shawhney, 1997). It is a single-gene recessive monogenic spontaneous mutation which has a pleiotropic effect on the *7B-1* phenotype. The mutation also changes the level of endogenous hormones and stress responses (Fellner and Sawhney, 2001; Fellner and Sawhney, 2002; Fellner *et al*., 2005; Bergougnoux *et al*., 2009; Bergougnoux *et al*., 2012). The *7B-1* mutant has an altered de-etiolazation and blue-light responses such as hypocotyl and epidermal cell elongation which suggests that the product of non-mutated *7B-1* gene is an element involved in the blue light signalling, likely in the phototropin pathway (Fellner and Sawhney, 2002; Bergougnoux *et al*., 2012; Hlavinka *et al*., 2013)

This work is based on a previous study where the altered responses of the *7B-1* mutant to an exogenous brassinosteroid were reported for the first time (Pilařová, 2014). The aim of this work was to test the *7B-1* growth responses to low concentrations of 24-*epi*brassinolide and to compare the expression levels of brassinosteroid receptor *BRI1* in the wild-type and *7B-1* hypocotyls under different light conditions.

The experiments were carried out in the Group of Molecular Physiology which is a part of the Laboratory of Growth Regulators at Palacký University Olomouc and Institute of Experimental botany AS CR. The work was supported by the Laboratory of Growth Regulators.

2 LITERATURE REVIEW

2. 1 Tomato mutant *7B-1*

2. 1. 1 Origin of mutant *7B-1*

The tomato (*Solanum lycopersicum* L.), a member of the *Solanaceae* family, is one of the most economically important crops. It is also very often used as model organism in plant research and therefore the functions of tomato genes are intensively studied. Using mutants with severe defects is one of the powerful tools for studying genes involved in molecular mechanisms of the particular signalling pathways.

The spontaneous recessive monogenic tomato mutant *7B-1* was isolated from cv. Rutgers background for its photoperiod-dependent male-sterility (Sawhney, 1997). Plants of the *7B-1* mutant grown under conditions of long day (LD, 16-h light/ 8-h dark, Figure 1A) were male-sterile. The flowers contained shrunken stamens (Figure 1D) which produced non-viable pollen; style and stigma were extruded from the corolla (Figure 1C). In short days (SD, 8-h light/ 16-h dark, Figure 1B), the plants were fertile with normal stamens and viable microspores, producing fruits containing seeds (Sawhney, 2004). The *7B-1* seeds are bigger, darker and heavier than the wild-type (WT) seeds (Fellner *et al*., 2001).

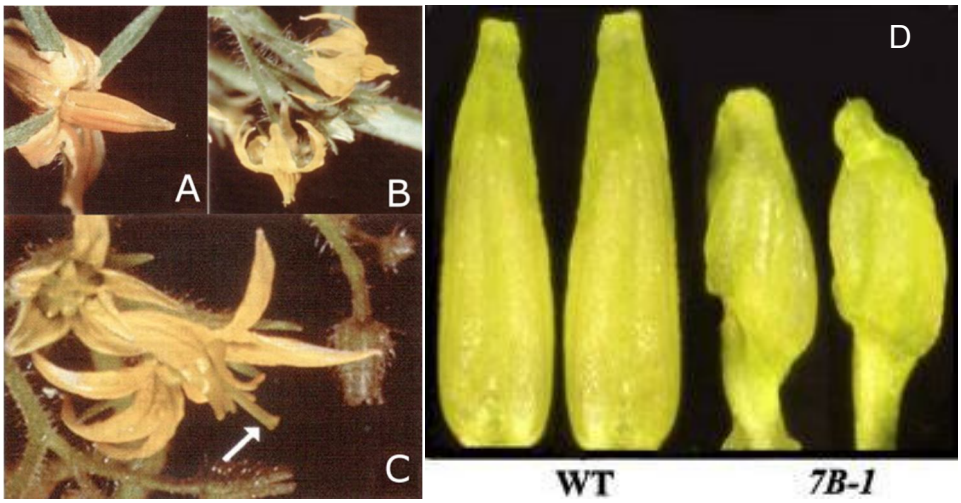


Figure 1: A: Wild-type (WT) flower grown in LD; B: *7B-1* flowers grown in SD with enclosed stigma; C: *7B-1* flowers grown in LD, arrow indicates extruded stigma (from Sawhney, 2004); D: Stamens from buds of WT and *7B-1* tomato grown in LD (from Sheoran *et al*., 2009)

According to the Sheoran's proteomic study, a large number of proteins, which have important roles in tapetum and microspore mother cells (MMCs), are differentially presented in the *7B-1* anthers during meiosis (Sheoran *et al*., 2009). One of the strongly up-regulated proteins in *7B-1* anthers is cystatin.

Similarly, other study focused on the regulatory role of miRNAs in the *7B-1* anther development showed that there are differences in the miRNA expression between WT and *7B-1* (Omidvar *et al*., 2015a). The strongest changes take place in the anther developmental stage where the MMCs are about to undergo meiosis. These

miRNAs guide the RNA-induced silencing complex, which causes a cleavage or transcriptional repression to their targets which have a potential role in microsporogenesis. Also, it was found out that the *7B-1* anther development is not synchronized among anthers. In some anther lobes, the newly formed microspores are not separated while in the others the microspores are free.

The wild-type and *7B-1* plants grown in a growth chamber do no differ in their height until approximately 65 days after seed sowing but later on, the *7B-1* plants are significantly taller (Figure 2) (Fellner *et al*., 2005).

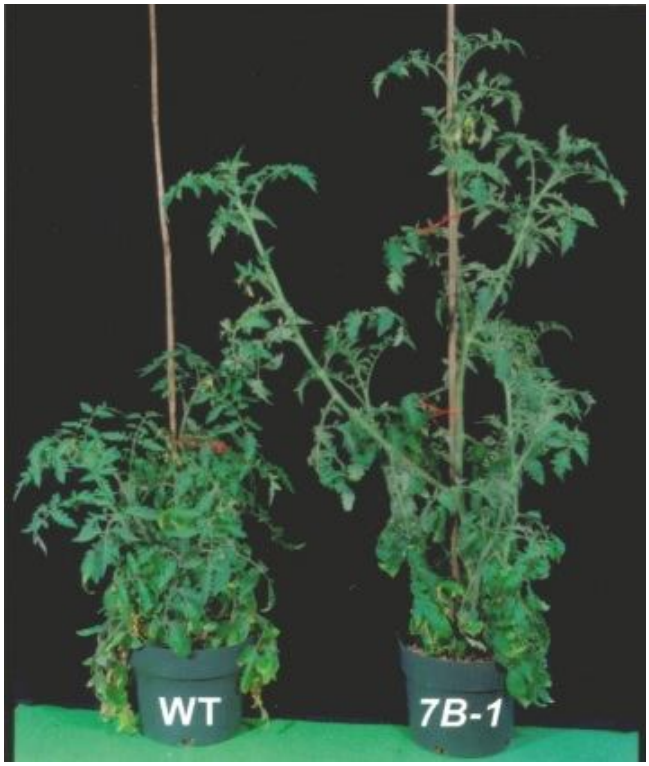


Figure 2: Three-months-old WT and 7B-1 plants grown under 16 h photoperiod (from Fellner *et al*., 2005).

2. 1. 2 Responses of *7B-1* mutant to abiotic and biotic stresses

Male-sterile tomato mutants are very useful for hybrid breeding programmes (Sawhney, 1997) but some of them can be sensitive to abiotic stress, which causes a limitation for their usage in the crop breeding. The *7B-1* mutant is the opposite case, according to the results obtained by Fellner and Sawhney the *7B-1* seeds are highly resistant to osmotic, salt and low temperature stress. Osmotic stress induced with 5% polyethylene glycol drastically affected wild-type germination, whereas the *7B-1* germination rate was almost four times higher (Fellner and Sawhney, 2001). Equally, the *7B-1* seeds were resistant to 100 mM mannitol and the same concentration caused 80 – 95% inhibition of the WT seeds. Similarly, the *7B-1* seeds were germinating much better in the presence of salts such as NaCl, KCl, Na_2SO_4 in the growth medium. The germination of WT seeds was strongly inhibited with the same salts. At optimal temperatures (25/23°C, light/dark), the germination rate of WT and *7B-1* was not different but the germination of *7B-1* seed was more tolerant to lower temperatures (16/13°C, light/dark) (Fellner and Sawhney, 2001).

In plants, the resistance to abiotic stress linked with the accumulation of abscisic acid (ABA), since its levels are known to be increased in stressed plants (Goldbach and Michael, 1976; Daie and Campbell, 1981; Mäntylä *et al*., 1995). In the light, especially in LD conditions, *7B-1* seeds were more sensitive to exogenous ABA than WT and the inhibitor of ABA biosynthesis, fluridone, suppressed the effect of low temperature on the germination rate more in the *7B-1* mutant (Fellner and Sawhney 2001, Fellner *et al*., 2001). Under blue light (BL), the *7B-1* seeds were strongly resistant to ABA relative to the WT (Fellner and Sawhney, 2002).

Moreover, the mutant seeds accumulate higher amount of nitric oxide, which is not only the antioxidant participating in the responses to stress caused by infection, but it also plays a positive role in the tomato seed germination induced by light, especially BL (Piterková *et al*., 2012). Nitric oxide is a part of the ABA-dependent pathway which controls stomata movement together with BL by inhibiting an unknown signalling component between phototropins and plasma membrane (Zhang *et al*., 2007).

Similarly, in BL the mutant *7B-1* had less symptoms and was less sensitive to a biotic stress induced by coronatine (COR), the phytotoxine, produced by *Pseudomonas syringae* strains, which causes alterations in plant metabolism (Bergougnoux *et al*., 2009).

2. 1. 3 Level of phytohormones in *7B-1* mutant

The data obtained during the germination experiments in abiotic stress conditions suggested that the *7B-1* seeds may have elevated level of ABA and that is why they can be more sensitive to exogenous ABA than the WT seeds are (Fellner and Sawhney, 2001). Moreover, the *7B-1* roots were ten times more sensitive to ABA than the WT roots in LD (Fellner *et al*., 2001). In LD, the WT shoots show higher transpiration rate than *7B-1* that could be also connected with higher level of ABA because the stomata closure induced by ABA was documented in many studies (e.g. Jones and Mansfield, 1970; Kriedemann *et al*., 1972).

The analysis of endogenous levels of phytohormones proved that the *7B-1* mutant is an ABA-overproducer. In contrast, the levels of indole-3-acetic acid (IAA) and gibberellins (GAs) were lower than in the control WT 7-day-old hypocotyls (Fellner *et al*., 2001).

Ethylene, other plant hormone, was studied in the *7B-1* mutant. The hypocotyl was more sensitive to ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in light, whereas the etiolated *7B-1* seedlings showed the same triple response (Figure 3) as WT (Fellner *et al*., 2005). The *7B-1* plants also produce a lower level of ethylene which is most likely connected with the elevated level of ABA (Fellner *et al*., 2005) because it is known that ethylene biosynthesis is affected by other plant hormones, including ABA (Spollen *et al*., 2000).

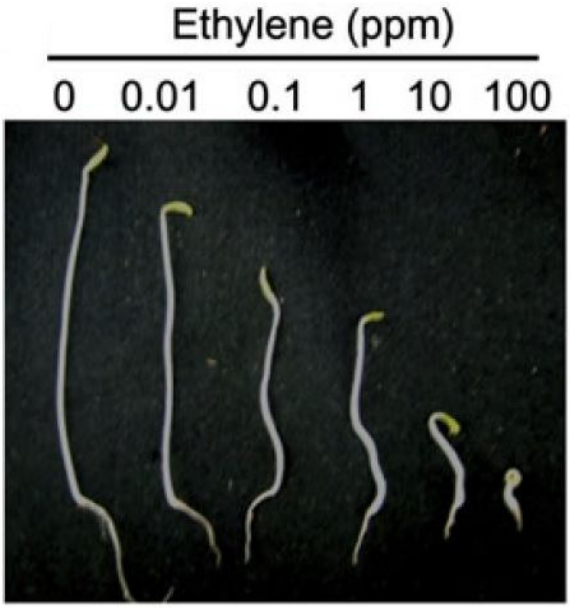


Figure 3: Triple response assay for exogenous ethylene in wild-type tomatoes after 6 days of germination in the dark (from Yokotani *et al.*, 2009).

Interesting results were also obtained in the case of another important group of phytohormones – cytokinins (CKs) which plays role in the establishment of photomorphogenesis in tomato (Bergougnoux *et al.*, 2012). The biggest difference between the *7B-1* and WT genotypes in the amount of CKs were in isopentenyladenine (iP). Whereas, its content was very low in the dark-grown seedling of both genotypes, BL induced a strong accumulation of iP in WT but not in *7B-1*.

The analysis of endogenous brassinosteroids (BRs) in the hypocotyls and roots grown in the dark, BL or RL showed that the mutant *7B-1* has five times lower concentration of brassinolide in the etiolated hypocotyls compared to WT (Pilařová, 2014). The *7B-1* and WT hypocotyls grown in RL and BL and the roots did not differ essentially in the amount of analysed BRs. Interestingly, neither the 10-day-old *7B-1* seedlings nor the WT showed altered hypocotyl growth response to tested concentrations of exogenous 24-*epi*-brassinolide in growth medium if they grew in the dark or RL. However, in blue light, 24-*epi*-brassinolide stimulated cell elongation and hypocotyl growth in the WT seedling. In BL and on the basal medium, the *7B-1* hypocotyl was longer than that in WT and its length was not affected by adding 24-*epi*-brassinolide in the medium (Figure 4; Figure 5).

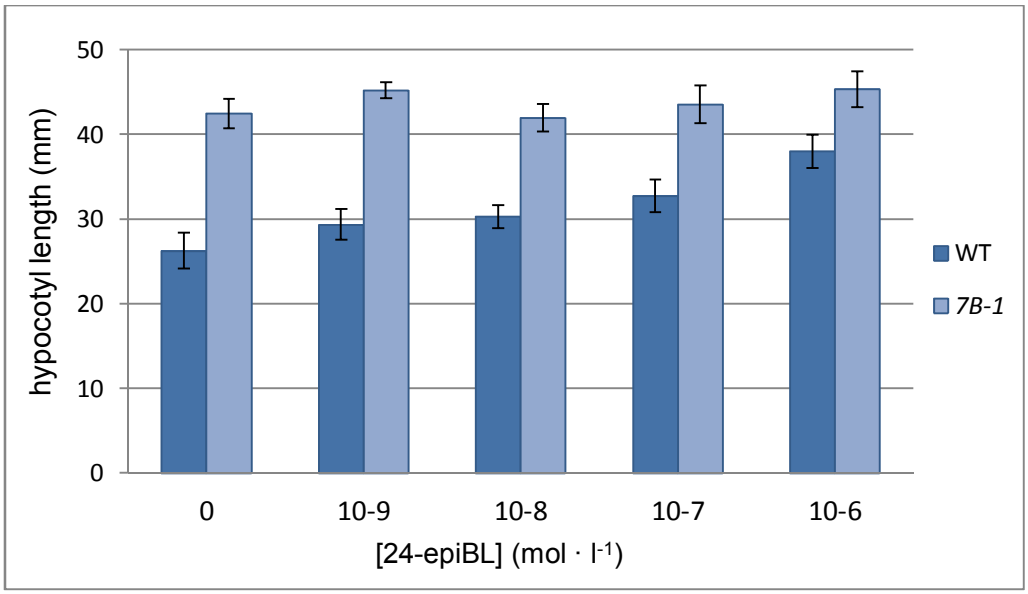


Figure 4: Effect of 24-*epi*-brassinolide on 10-day-old-hypocotyl length of *Solanum lycopersicum* L. cv. Rutgers (wild-type, WT) and mutant *7B-1* grown in continuous blue light. Values represent mean ± SE (n = 4) (from Pilařová, 2014).

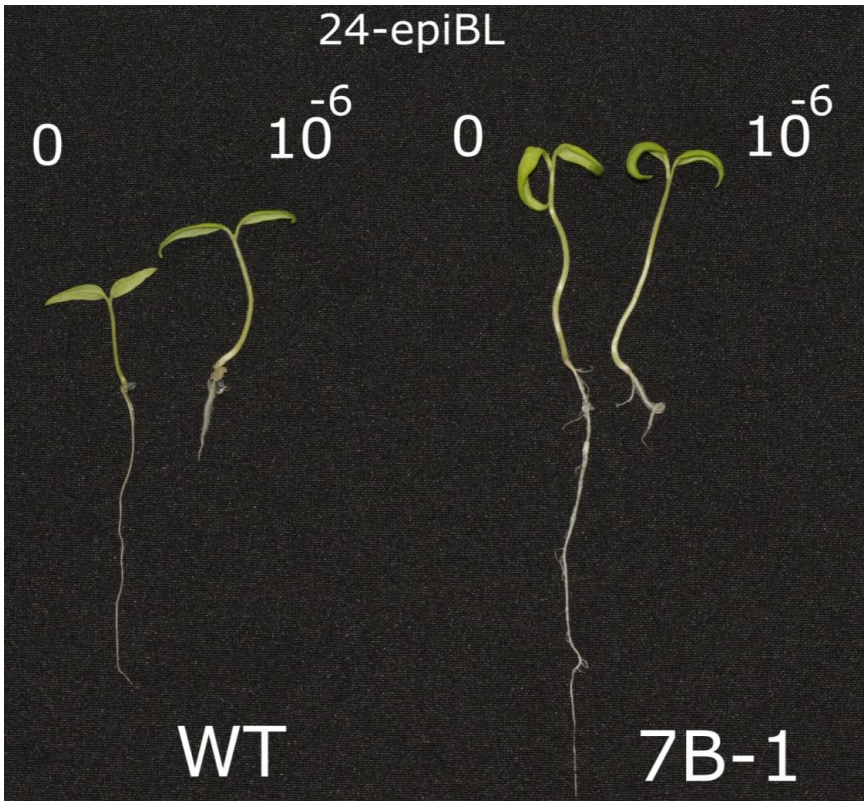


Figure 5: Effect of 24-*epi*-brassinolide on phenotypes of 10-day-old tomatoes *Solanum lycopersicum* L. cv. Rutgers (wild-type, WT) and mutant *7B-1* grown in continuous blue light (from Pilařová, 2014).

2. 1. 4 Responses of *7B-1* mutant to light

Inhibition of the WT seeds germination by abiotic stresses was stronger in the light than in the dark (Fellner and Sawhney 2001). Interestingly, pronounced difference between the *7B-1* and WT in the ability to overcome abiotic stress and germinate was observed

only in the light, especially in white or BL but not in red light (RL) (Fellner and Sawhney 2002).

The light-induced reduction of hypocotyl growth in *7B-1* mutant is noticeable less than in WT in LD condition, and it was observed that the *7B-1* epidermal cells are longer than those in WT (Fellner *et al.*, 2001, Bergougnoux *et al.*, 2012). In the dark and RL, the hypocotyls of WT and *7B-1* mutant do not show any differences in their length, however, the *7B-1* hypocotyls were much less de-etiolated compared to WT in BL (Fellner and Sawhney 2002). Based on these results, Fellner and Sawhney first formulated the hypothesis that *7B-1* gene is involved in BL reception or signal transduction.

One of the processes tightly connected with photomorphogenesis is the endoreduplication which is characterized by chromosomal DNA synthesis without mitosis and cytokinesis (Traas *et al.*, 1998). In the tomato hypocotyls developed under continuous BL, there is more of 2C cells and less 4C than in the dark grown hypocotyls but the *7B-1* mutant contains higher portion of 4C cells and lower of 2C (Bergougnoux *et al.*, 2012).

An obvious question was whether photosynthesis and transpiration are somehow altered in the *7B-1* mutant. The measurements of the net photosynthetic rate (P_N) and stomatal conductance (g_s) suggested that the *7B-1* leaves distinctly differ from those in WT if they are older and if they grow under BL (Ježilová *et al.*, 2012). Detected P_N and g_s were slower increased by light in the *7B-1* leaves compared to WT. In contrast, the intrinsic water-use efficiency (WUE_i) was higher in *7B-1* genotype. These dissimilarities are probably connected with the elevated ABA level (Ježilová *et al.*, 2012) as it is known that WUE_i rises with the increasing endogenous ABA level (Zhang *et al.*, 2004).

Hlavinka *et al.* examined the BL-induced stomata opening in the *7B-1* mutant. They revealed that *7B-1* gene is somehow involved in the early BL-induced signalling pathway involved in stomatal opening because the younger plants of the *7B-1* genotype were almost resistant to BL-opening and in the 5-week-old *7B-1* plants, the stomatal aperture was reduced (Hlavinka *et al.*, 2013).

Several facts leads to conclusion that *7B-1* mutant is not affected in cryptochrome 1 (Sheoran *et al.*, 2006, Omidvar and Fellner 2015) but it may be affected in phototropin signalling pathway because *7B-1* mutant shows defects in other phototropin-specific responses (Bergougnoux and Fellner, unpublished data).

2. 1. 5 Role of *7B-1* mutation in accumulation of pigments

The accumulation of photosynthetic pigments also differs between WT and the *7B-1* mutant. The chlorophyll content in the mutant hypocotyls and leaves is higher compared to WT (Fellner *et al.*, 2005, Ježilová *et al.*, 2012). In contrast, the accumulation of anthocyanins, secondary metabolites of the flavonoids biosynthetic pathway, is reduced in the *7B-1* mutant and it is likely connected with the ABA overproduction as well (Sheoran *et al.*, 2006). The lower activity of phenylalanine ammonia-lyase (PAL), which is an enzyme involved in the anthocyanin biosynthesis, was reported in the *7B-1*, even though there was not any alteration in the expression of PAL. This implies that the anthocyanin accumulation is regulated at the posttranslational level and it could be one of the factors that control male-sterility in the *7B-1* mutant.

2. 1. 6 Changes in gene expression level in the *7B-1* mutant

Several studies examined interesting changes in expression levels of various genes in the *7B-1* mutant (Sheoran *et al*., 2006; Bergougnoux *et al*., 2009; Sheoran *et al*., 2006; Omidvar *et al*., 2015a; Omidvar *et al*., 2015b, Omidvar and Fellner, 2015).

Whereas the quantification of *COI1* (*CORONATINE-INSENSITIVE1*) gene expression, which is involved in jasmonic acid (JA) signalling, did not show any difference between the two genotypes, the expression pattern of *NPR1* (*NON-EXPRESSOR OF PATHOGENESIS-RELATED1*) gene involved in salicylic acid (SA) signalling pathway revealed that the *7B-1* hypocotyls contained more of the *NPR1* transcript than those in WT (Bergougnoux *et al*., 2009). The difference was noticeable in the dark and BL.

The expression of the key regulators of pollen cell wall and pollen tube development, pectinmethylesterase (PME) and pectinmethylesterase inhibitor (PMEI), was altered in the *7B-1* anthers (Omidvar *et al*., 2015b). *PMEI* is up-regulated and it may suppress the PMI activity and impaired the enzymatic degradation of pectin. It could consequently lead to the failure of the proper separation of tetrades in the *7B-1* anthers. Omidvar *et al.* also created two mRNA libraries from *7B-1* and WT tomato seedlings grown in BL and under abiotic stress conditions (Omidvar *et al*., 2015a). There were identified miRNAs which regulate expression of several genes that might be associated with the enhanced tolerance of the *7B-1* mutant to abiotic stress under BL.

A crucial finding for the exploration of the *7B-1* mutant was also the fact that BL receptors (cryptochromes and phototropins) are not affected by the *7B-1* mutation at transcriptional level, but the mutation most probably affects a down-stream component of the BL signalling pathway (Sheoran *et al*., 2006; Omidvar and Fellner, 2015). Omidvar and Fellner (2015) concluded that DNA methylation remodelling is an active epigenic response to abiotic stresses and showed that there are significant differences in the genomic methylation level in dark-grown stressed *7B-1* and WT seedlings. There was not any clear difference between the BL-grown seedlings.

Two experiments studying the expression of gene coding brassinosteroid receptor BRI1 (BRASSINOSTEROID INSENSITIVE1) in the WT and *7B-1* hypocotyls were carried out (Pilařová, 2014). The results suggested that BL may affect *BRI1* expression level in both genotypes and the *7B-1* mutant could express less *BRI1* in the dark than WT. The level of *BRI1* transcript was established based on the intensity of band obtained by semiquantitative reverse transcription PCR and the expression was not related to any housekeeping gene.

2. 2 Light perception and signalling in plants

Light is electromagnetic radiation which is one of the main environmental stimuli for plants and it controls two very important processes – photomorphogenesis and photosynthesis. Whereas in photosynthesis light serves as the source of energy for green plants, in photomorphogenesis it represents a signal which causes switching from development in the dark – skotomorphogenesis – to development in light. Light

perception is provided by several types of photoreceptors which have a specific range of wavelengths that they are able to recognise.

2. 2. 1 Red light

Phytochromes recognise red and far-red part of the light spectrum and control an essential processes in plants e.g. photoperiodism, flowering, leaf expansion and seed germination (Parihar *et al*., 2016). In low amount, they also absorb blue light (Parihar *et al*., 2016). Based on experiments with lettuce seeds which germination is photoreversible, two conformations of phytochromes were proposed – inactive red light absorbing form Pr and active far-red absorbing Pfr (Borthwick *et al*., 1952).

In *Arabidopsis thaliana*, five phytochromes (Phy A–E) have been identified (Sharrock and Quail, 1989). Their chromophores are open-chain tetrapyrroles; the phytochrome's *N*-terminal part is the input site for the signal and the *C*-terminal end represents the output part (Parihar *et al*., 2016). Li *et al*. (2010) found out that phytochrome proteins build homodimers.

Phytochromes change their localisation in the cell; after light irradiation they move from the cytoplasm into the nucleus where directly or indirectly activate the transcription (Kircher *et al*., 2002). Studies reported that PhyA and Phy B interacts with transcription factor PIF3 (Phytochrome Interacting Factor 3) and basic helix-loop-helix (bHLH) (Ni *et al*., 1998). Under light irradiation, PhyA co-localises with the central regulator of light signalling E3 ubiquitin ligase COP1 (CONSTITUTIVE PHOTOMORPHOGENESIS 1) in the nucleus and their interaction was shown in-vitro by Seo *et al*. (2004).

2. 2. 2 Blue light

The blue part of light spectrum is detected in plants by three types of photoreceptors: cryptochromes, phototropins and the most recently discovered Zeitlupe family (Parihar *et al*., 2016).

Cryptochromes are blue/UV-A receptors (Parihar *et al*., 2016). Cryptochrome 1 (cry1) was described thanks to the *Arabidopsis hy4* mutant, which had a defect in *CRY1* and showed elongated hypocotyl in white and blue light only. In the dark, *C*RY1 is localised in the nucleus and upon irradiation in the cytoplasm. Other two cryptochrome isoforms are known to be in *Arabidopsis thaliana.* It was suggested that cryptochrome require dimerization for their functionality (Yang *et al*., 2000).

Phototropism, chloroplast movement and stomatal opening are regulated by blue light through the plasmamembrane associated receptors – phototropins (Parihar *et al*., 2016). The parts of photropin that can perceive light are two N-terminal light, oxygen and voltage domains – LOV1 and LOV2 which regulate cofactor binding and contain flavinmononucleotide as phototropin chromophore. After irradiation phot1 and phot2 are autophosphorylated which is dark-reversible process (Christie *et al*., 1998). Two types of phototropins are known: phot1 and phot2; which were originally called NPL1 and NPH1 (Kagawa *et al*., 2001).

LOV domain is also part of Zeitlupe photoreceptor molecule, the last group of blue light photoreceptors (Parihar *et al*., 2016). Zeitlupes are localised in cytosol and nucleus and their chromophore is also flavinmononucleotide. They influence mainly circadian clock (Mas *et al*., 2003).

2. 3 Brassinosteroids

Brassinosteroids (BRs) are a group of phytohormones with a polyhydroxysteroid structure (Figure 6) similar to the animal steroid hormones (Grove *et al.*, 1979). The first part of their name comes from *Brassica napus* L. because brassinosteroids were isolated from its pollen and the compound with the highest bioactivity was named brassinolide (Mitchell *et al.,* 1970).

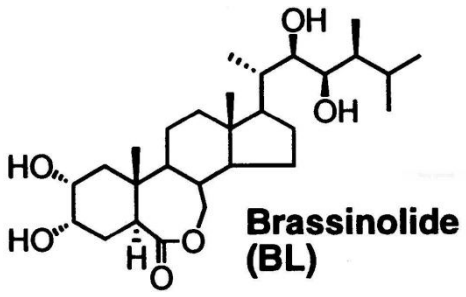


Figure 6: Brassinosteroid chemical structure represented by the most bioactive member of the group – brassinolide (from Noguchi et al., 2000).

2. 3. 1 Bioactivity of brassinosteroids

The brassinosteroid bioactivity could be studied thanks to BR-deficient and BR-insensitive mutants which had been identified in *Arabidopsis* (Clouse *et al*., 1996; Li *et al*., 1996; Szekeres *et al*., 1996). BR-deficient mutant *de-etiolated-2* (*det2*) (Figure 7) and *constitutive photomorphogenesis and dwarfism* (*cpd*) mutants showed strong fenotype i.e. presence of photomorphogenesis in the dark and extreme dwarfism, respectively. It suggested the molecular pathways where BRs plays an essential role: light-regulated development, cell elongation, seed germination, stomata formation, flowering and fertility and vascular development (Wang *et al.,* 2012).

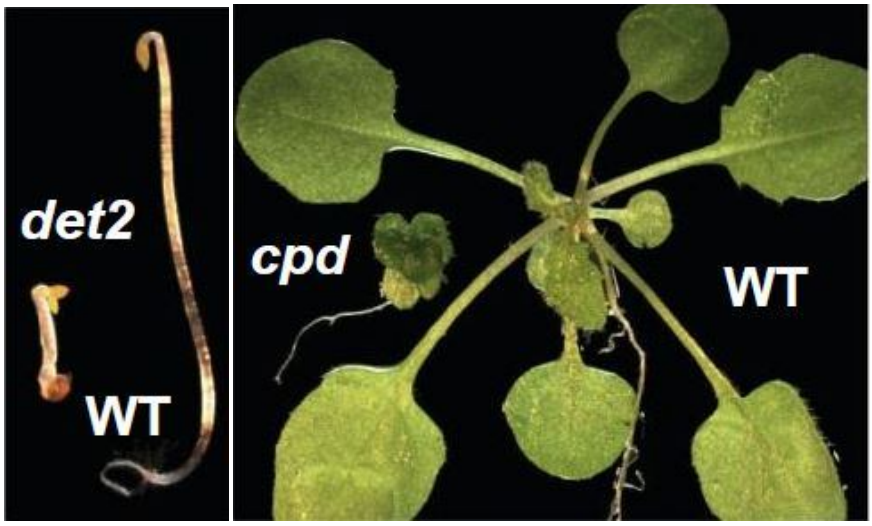


Figure 7: Fenotypes of brassinosteroid-deficient *Arabidopsis* mutants; *de-etiolated-2* (*det2*) and wild-type (WT) plant grown in the dark (left), *constitutive photomorphogenesis and dwarfism* (*cpd*) and WT (right) (from Taiz and Zeiger, 2006)

Brassinosteroids have a large effect on the plant growth and development even in very low concentrations (Wang *et al.,* 2012). To compare the bioactivity of different BRs,

several bioassays were developed, for example the rice lamina inclination test (Wada *et al.,* 1981) (Figure 8) and the bean second internode bioassay (Thompson *et al.*, 1981) (Figure 9).

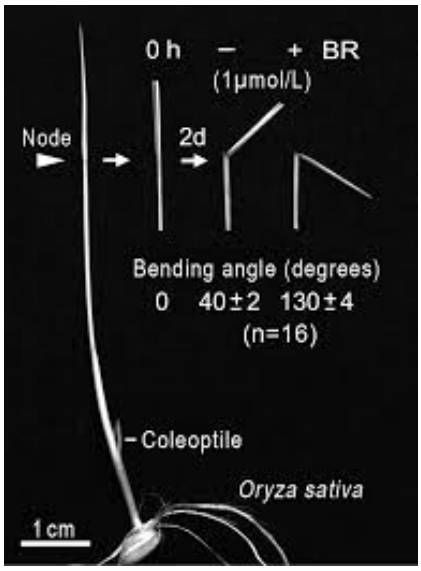


Figure 8: The effect of brassinosteroid on *Oryza sativa* in the rice lamina inclination bioassay (from Kutschera and Wang, 2012)

Figure 9: The bean second internode brassinosteroid bioassay. First, BR promotes elongation. In the higher concentration stimulates cell expansion and the highest concentration causes splitting of the internode (from Taiz and Zeiger, 2006).

2. 3. 2 Brassinosteroid signalling

Brassinosteroid signalling pathway was studied extensively. The *Arabidopsis* dwarf mutant named *brassinosteroid insensitive 1 (bri1)* (Figure 10) was isolated and the studies suggested that the mutation affects receptor kinase BRI1 which is crucial for the BR-response (Clouse *et al.,* 1996; Li and Chory, 1997).

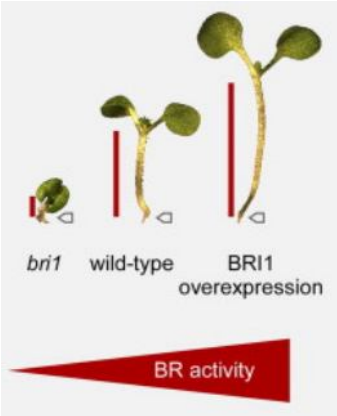


Figure 10: Phenotypes of *Arabidopsis* mutant *bri1* and BRI1 overexpression seedling (from Fridman and Savaldi-Goldstein 2013).

It was shown that BRI1 is membrane localised BRs receptor with 25 leucin-rich repeats (LRRs) arranged into a super helix shape on the extracellular domain (Wang *et al.,* 2001; Hothorn *et al*., 2011). Between the LRR21 and LRR22 there is a 70-amino acid island which forms BR binding site (Kinoshita *et al.,* 2005*;* She *et al.,* 2011). The ligand binding does not cause overall conformational change in the extracellular domain backbone, it just creates a hydrophobic surface that may provide a docking platform for unknown protein. Upon BR binding, BRI1 gains its kinase activity and it can interact with co-receptor kinase BAK1 (BRI1-ASSOCIATED RECEPTOR KINASE1) (Wang *et al.,* 2005; Wang and Chory, 2006). The data supported the hypothesis that there is a sequence of transphosphorylation between BAK1 activation loop and BRI1 juxtamembrane domain to enhance BRI1 signalling (Wang *et al*., 2008; Clouse 2011). Inactive BRI1 associates with BKI (BRI1 KINASE INHIBITOR) and prevent BAK1 binding (Li *et al.,* 2002; Nam and Li, 2002). The presence of BRs causes dissociation of BKI from BRI1 and from plasma membrane which is probably connected with BKI's interaction with 14-3-3 protein.

BRI1 phosphorylates two subclasses of RLCKs (receptor-like cytoplasmic kinases): BSK (BR/SIGNALING KINASE) and CDG1 (CONSTITUVITE DIFFERENTIAL GROWTH1) (Tang *et al.,* 2008; Kim *et al.,* 2011). They activate BSU1 (BRI1-SUPRESSOR1) which is autophosphorylated and causes BIN2 (BRASSINOSTEROID-INSENSITIVE 2) degradation (Kim *et al.,* 2009) (Figure 11).

BIN2 is a negative regulator and it is active in the absence of BRs (Figure 11 A) (He *et al.,* 2002). It causes inactivation of transcription factors BRZ1 and BES1/BRZ2 which leads to their proteosomal degradation (He *et al.,* 2005; Sun *et al.,* 2010; Yin *et al.,* 2002; Yin *et al.,* 2005). In the presence of BR ligand (Figure 11 B), BIN2 is degraded, BRZ1 and BES1/BRZ2 are massively dephosphorylated by phosphatase 2A (PP2A), so they can move to the nucleus to bind genomic DNA and regulate gene expression.

BRZ1 and BES1/BRZ2 target genes have a function in the BR biosynthesis pathway and BR signalling (Wang *et al.,* 2012). Other genes regulate cell wall synthesis, cytoskeleton, inhibition of chloroplast development and photomorphogenesis. A big group of BRZ1 and BES1/BRZ2 target genes are involved in interactions pathways with other phytohormones as GAs, auxin or ABA.

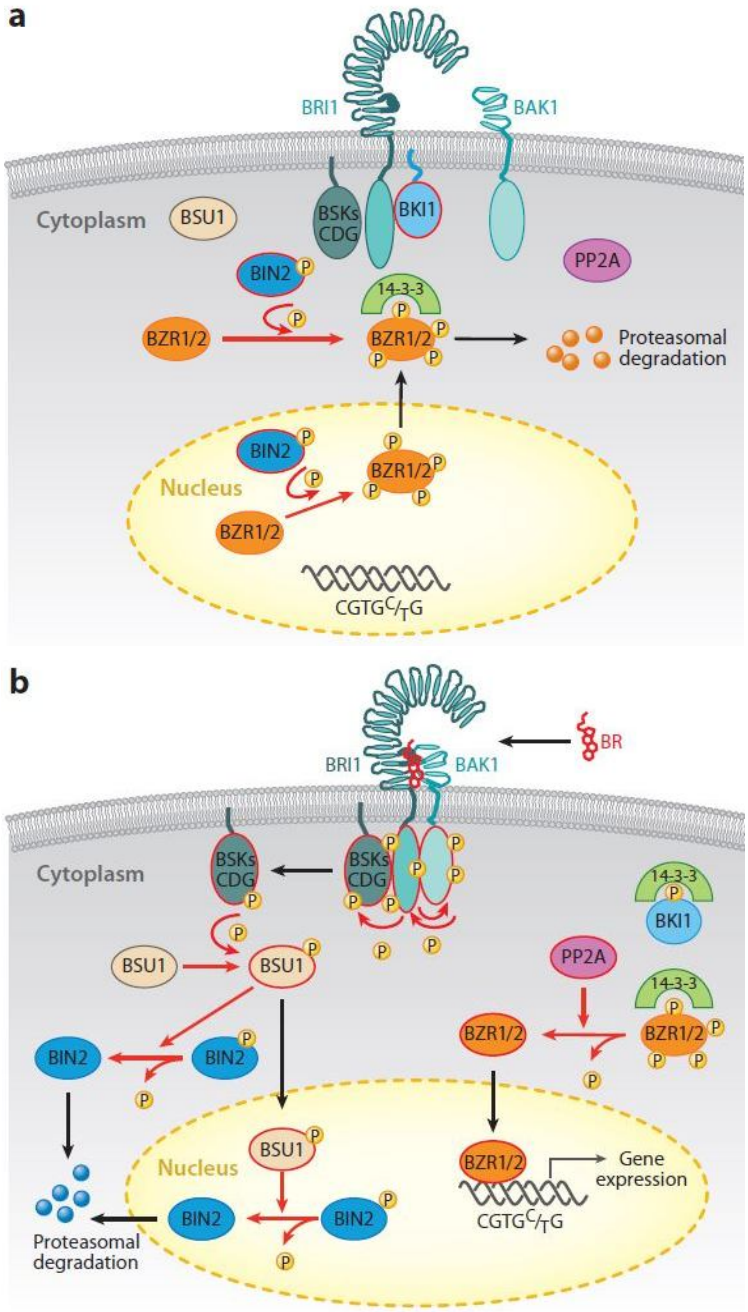


Figure 11: Brassinosteroid signal transduction pathway. A) In the absence of brassinosteroid (BR); B) In the presence of BR. Active components are marked by red outlines, P and red arrows means phosphorylation or dephosphorylation, black arrows indicate protein movement (Wang *et al.,* 2012).

The functional analysis of tomato BRI1 and BAK1 showed that they are active in autophosphorylation and they can also transphosphorylate each other as well as BRI1 and BAK1 in *Arabidopsis* (Bajwa *et al.*, 2013).

2. 4 Interaction of light and BRs pathways

Brassinosteroids and light play very important roles in many similar physiological processes such as photomorphogenesis, elongation, flowering or shade-avoidance, and therefore they have to somehow interact or to be connected (Wang *et al.,* 2012) (Figure 12). As it was described above, BR-mutant's phenotype in the dark is similar to

WT phenotype in light (Li *et al*., 1996). WT plants treated with BR inhibitors grown in the dark have short hypocotyls (Clouse and Sasse, 1998).

Plants react to changes of environment by adjusting the levels of phytohormones and light is one of the environmental factors. Some studies identified several cytochromes P450 that inactivate or degrade BRs, e.g. BAS1 (Neff *et al*., 1999), SOB7 (Turk *et al*., 2005) or CYP72B1 (Turk *et al*., 2003). Symons *et al*. (2008) did not measure any significant difference in endogenous BRs levels between plants growing under continuous light and dark. Luo *et al.* (2010) did not notice any change in BZR1 activation between dark-grown and light-grown A*rabidopsis* seedlings. It means that light does not influence BR signalling pathways through enhancing the activity of the BZR1 transcriptional factor (Wang *et al.*, 2012). However, in some other plant species, higher BR levels were found if they grew in light, e.g. peas seedlings in light had a higher expression of the *DWF4* gene, which is involved in BRs synthesis (Symons *et al.,* 2002).

Microarray experiments suggested that there is a relationship between light-regulated genes and genes affected by BZR1. Many genes encoding the components of light signalling pathways are also targets for BZR1, e.g. PIF4 (Oh et al., 2012, Bai *et al*., 2012). BRs regulate transcription of the light-signalling components and decrease the plat response to the light signal (Wang *et al*., 2012). Kim *et al*. (2014) suggested that light may control BRs signalling through COP1 which degrades BZR1 in the dark.

Another key transcriptional regulator that mediates crosstalk between BR and light signalling is called GATA2 (Luo *et al*., 2010). GATA2 directly regulates genes that respond to BR and light and which are involved in de-etiolation. BRs repress expression of GATA2 through transcriptional factor BZR1. Light causes accumulation of GATA2 through the inactivation of COP1 which ubiquitinates GATA2 and marks it for proteasomal degradation.

Brassinosteroids also play a role in shade-avoidance responses which is a response of plants in dense vegetation where the growth can be affected by light competition with the neighbours (Keuskamp *et al*., 2011). The proximity of other plants causes the reduction in the red/far-red ratio controlled by phytochromes and it is depended on auxin biosynthesis but it can be also induced by blue light (Keller *et al*., 2011). The BR response is required to elicit the phenotype of shade-aviodance under blue light as well as PIF4 and PIF5. Under blue light, the response is fully inhibited only if auxin and BR are blocked (Keuskamp *et al*., 2011). These hormones both regulate cell elongation through *XTH* (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDRO-LASE) genes. The activation of phyA and cry1 leads to the activation of enzyme ROC1 (ROTAMASE CYCLOPHILIN1) which inhibits BES1 (Trupkin *et al*., 2012; Vriet *et al*., 2013).

Blue light is involved in rice lamina bending and unrolling (Asahina *et al*., 2014). It is mediated via castasterone, an endogenous BR, which biosynthesis is enhanced in BL. The analysis of A*rabidopsis* mutant *elg* (elongated) showed that BRs also influence another BL specific reaction – phototropism, through BAK1 (Whippo and Hangerter, 2005).

Choi *et al.* (2014) used iNID (*N*etwork models delineating *I*nterplays among *D*evelopmental signalling) to investigate the auxin–BR–blue light interplays and they experimentally verified two of them: BME3 and TEM1, which modulate germination and flowering.

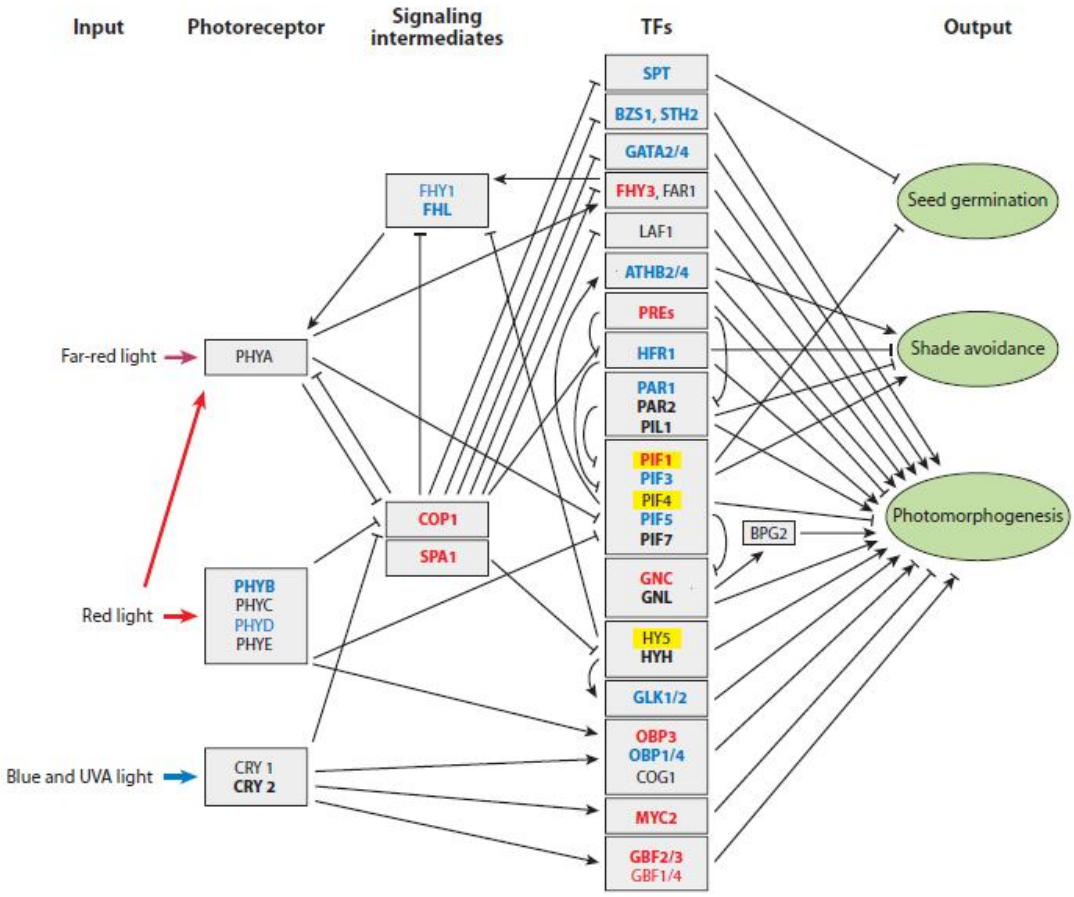


Figure 12: Schematic summary of BR and light-signalling pathways which control physiological responses. Arrows indicate activation, bar-ended lines inhibition; BR-activated and BR-repressed genes are in red and blue, respectively; TFs – transcription factors (from Wang *et al.,* 2012).

3 MATERIAL AND METHODS

3. 1 Plant material

Solanum lycopersicum L. cultivar Rutgers (wild type, WT) and its mutant *7B-1* were used as plant material for this study. The mutant *7B-1* shows male sterility depending on the length of the photoperiod. Pollen grains of the *7B-1* mutant plants growing under long day conditions (LD; 16 hours of light / 8 hours of dark) are often defective resulting in *7B-1* sterility. In contrast, *7B-1* plants grown in short day conditions (SD; 8 hours light / 16 hours dark) are fertile (Sawhney, 1997; Omidvar *et al.*, 2015).

The tomato mutant *7B-1* is not fully genetically characterised but it is known that the *7B-1* phenotype is caused by spontaneous mutation which is recessive and monogenic (Sawhney, 1997). Previous studies suggest that the functional product of the *7B-1* gene is somehow involved in the blue light signalling pathway (Fellner and Sawhney, 2002; Bergougnoux *et al.*, 2009; Hlavinka *et al.*, 2013).

The seeds of both genotypes were provided by Prof. V. K. Sawhney based on the licence from the University of Saskatchewan, Saskatoon, Canada.

3. 2 Seed sterilisation

The seeds of each genotype were surface sterilised in plastic tubes (maximum volume 50 mL) by soaking in approximately 30 mL of 3% sodium hypochlorite (Savo, Bochemie a. s., Czech Republic) and shaking continuously for 25 minutes. Then, the solution was removed and the seeds were extensively rinsed in 30 mL of sterile distilled water. The last step was repeated five times.

3. 3 Medium preparation

Solid basal MS medium (Murashige and Skoog, 1962) was used as the growth medium in all experiments. It was prepared using the following procedure:

Sucrose (20.0 g, Lachner, cat. n. 40135-APO), MS medium powder (8.6 g; Caisson LABS, US, cat. n. MSP01-50LT) and MES (Morpholinoethane sulfonic acid; 390.4 mg; Serva, cat. n. 29834.04) were dissolved in 1 L of distilled water. Distilled water was added to reach the total volume of 2 L. After that, the pH value of the solution was adjusted to 6.1 by 1M solution of potassium hydroxide.

The solution was well mixed and divided into five pyrex bottles (capacity 500 mL). To each bottle, 2.8 g of agar (Duchefa Biochemie, cat. n. P1001.1000) were added. The caps were covered with an aluminium foil and the bottles with the medium were sterilized in an autoclave. The pH value of the medium after autoclaving decreased approximately to 5.7. After cooling off, the bottles containing the solid medium were stored at 4°C. Before using, the medium was melted in a microwave oven for a few minutes and poured into the plastic Petri dishes (diameter 90 mm, 20 mL of medium in each) under sterile conditions in a laminar hood.

3. 4 Brassinosteroid treatment

For the experiments on brassinosteroid treatment, the required amount of 24-*epi*-brassinolide (24-*epi*-BL; Mr = 480.68) stock solution was added into the cold liquid medium. The stock solution (c = 10^{-3} M) was prepared by dissolving powdered 24-*epi*-BL in 99.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, cat. n. 066K0173) and distilled water was added to final volume. The final concentration of DMSO in the stock solution was 25 vol. %.

In all experiments studying *BRI1* gene expression which included brassinosteroid treatment, 10^{-6} M concentration of 24-*epi*-BL was used. In the last experiment which included brassinosteroid treatment, a proper control was prepared by adding 99.5% DMSO into the medium to reach the same final concentration of DMSO (0.025 vol. % in the medium).

The 10^{-6} M concentration of 24-*epi*-BL was chosen because of the results from previous experiments. These experiments showed that growth of hypocotyl and epidermal cell elongation in WT tomato seedlings incubated under blue light was stimulated by 10^{-6} M 24-*epi*-BL added to the medium (Pilařová, 2014). In the experiments studying growth responses four different concentrations were included: 10^{-9} M, 10^{-10} M, 10^{-11} M and 10^{-12} M concentration of 24-*epi*-BL in MS medium.

The 24-*epi*-brassinolide was provided by Dr. Jana Oklešťková from the Laboratory of Growth Regulators at Palacky University in Olomouc, Czech Republic.

3. 5 Growing conditions

The sterilized seeds were sown on the solid basal MS medium in Petri dishes. The Petri dishes were twice wrapped around with textile tape SoftPore (BATIST s. r. o.), covered in aluminium foil and placed vertically in a growth chamber (Microclima 1000E, Snijders Scientific B.V., The Netherlands) at a stable temperature of 23°C for 3 or 4 days to induce germination.

The germinated seeds were transferred on fresh MS medium supplemented or not with 24-*epi*-BL or a corresponding amount of DMSO (for last experiment including 24-*epi*-BL treatment). The number of seeds was 7 seeds per each Petri dish. The transfer was performed in sterile conditions under white light. The dishes were wrapped around with textile tape and placed vertically in the growth chamber with continuous blue light illumination or covered in aluminium foil (for growth in the dark) at a stable temperature of 23°C. Blue or red light illumination was provided by fluorescent tubes (TL-D36W/18-Blue and TLD36W/15-Red, Philips, respectively). The tomato seedlings were grown under these conditions for 3 or 7 days depending on the type of experiment.

3. 6 Measurement of hypocotyl and root length

The plants were first visually evaluated and then, they were manually measured on a graph paper with a ruler. The lengths were measured with 1 mm accuracy and the mean ± standard error (SE) values were calculated.

3. 7 Hypocotyl harvesting

The upper half of the 3 or 7-day-old tomato hypocotyls were harvested under blue light or green light (for etiolated seedlings). The cotyledons were cut off with a scalpel, and approximately 200 mg of hypocotyl tissue for each growth condition and genotype were packed in aluminium foil and immediately frozen in liquid nitrogen. The harvested samples were stored at -80°C until grinding.

3. 8 RNA extraction

The frozen samples were manually grinded in sterilized mortars which had been cooled down in liquid nitrogen. The fine powder was transferred to micro-centrifuge tubes (volume 2 mL) and stored in a freezer (-20°C) until all samples were grinded.

For RNA extraction and purification, the Isolate II RNA Plant Kit (Bioline, UK) was used. The basic procedure of the used kit is shown in Figure 13.

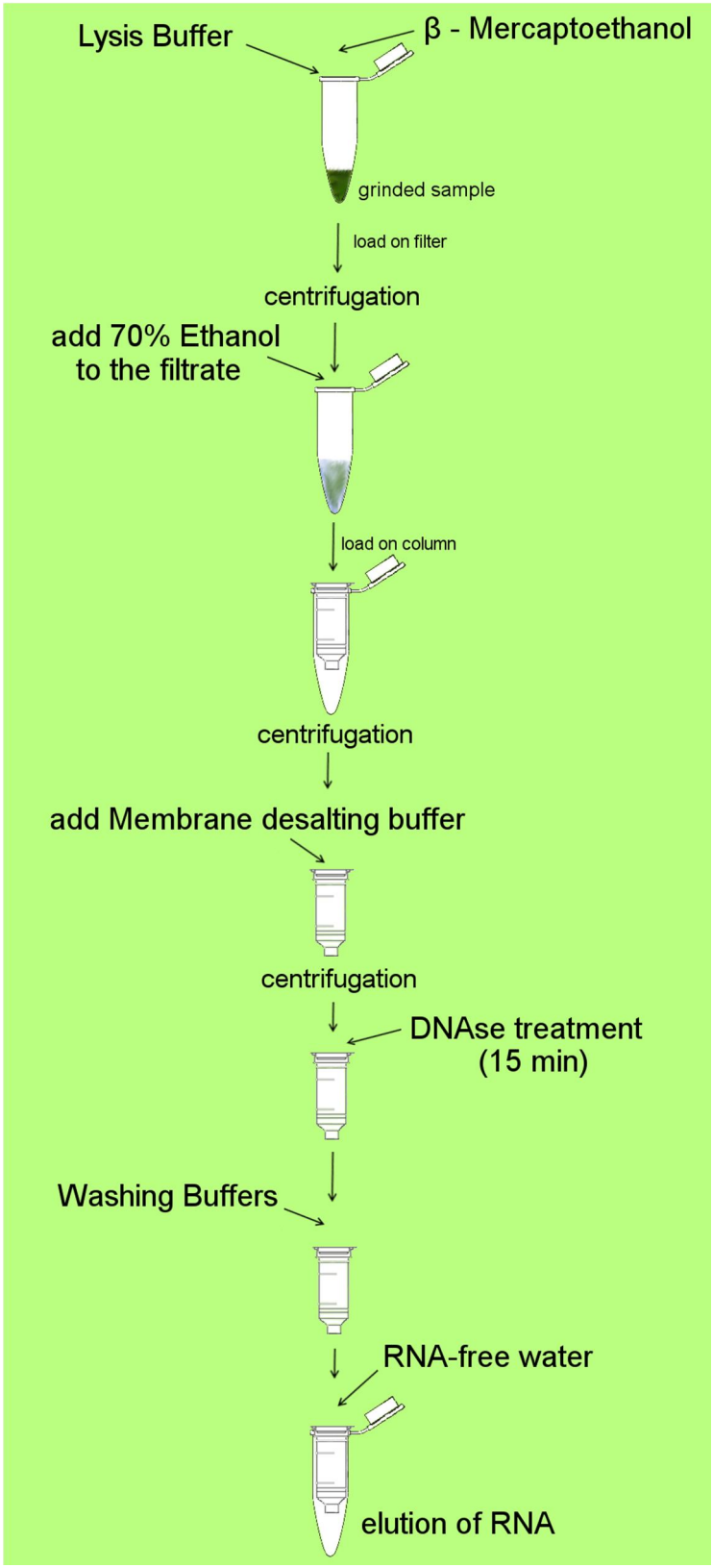


Figure 13: The basic procedure of RNA extraction using Isolate II RNA Plant Kit (Bioline, UK).

The concentration of RNA was measured on spectrophotometer SmartSpec Plus (Bio-Rad, US) and the purity of RNA was checked by polymerase chain reaction (PCR) using following primers and procedure:

Reaction mixture:

4.0 µL of 5× My Taq Buffer (Bioline, UK)

0.5 µL of each primer:

LeGAPDH-F primer: 5´ – ATC CAT TTA TTT CCA CTG ATT ACA A – 3´

LeGAPDH-R primer: 5´ – CAT GGG AGC ATC TTT GCT A – 3´

0.1 µL of My Taq Polymerase (Bioline, UK)

2.0 µL of isolated RNA sample as a template

RNA-free water up to final volume (20 µL per reaction)

Program:

94°C	3 min	
94°C	30 s	45 cycles
55°C	30 s	
72°C	1 min	
72°C	5 min	
15°C	∞	

Gene *LeGAPDH* encodes tomato glyceraldehyde-3-phosphatedehydrogenase (accession number: U97257; Chen, 1997) and it could be amplified during the reaction only if the sample contained DNA. The presence of amplified product was checked by performing horizontal electrophoresis in 1.5% agarose gel containing GelRed Nucleic Acid Stain (cat. n. 41 003, Biotium, US). Samples were mixed with 6× loading Dye (Biogen, US) before loading on a gel. The PCR products in gel were visualised by Gel DOC EZ Imager (BioRad, US).

In the case, the RNA samples were contaminated by genomic DNA, the Dnase treatment in solution followed. DnaseI and Reaction Buffer for DnaseI (both Bioline, United Kingdom) were added into the RNA samples. This mixture was incubated for 10 min at 37°C and after that sodium acetate (3M, pH 5.2) and 96% ethanol was added. Samples were precipitated overnight at -80°C. After that, they were centrifuged for 10 min at 4°C at maximum speed and the supernatant was removed by inversing the tube. RNA pellet was washed with 70% and then 96% ice-cold ethanol and then the tubes were open to let RNA pellet dry out. RNA was then dissolved in RNase-free water and its purity was checked once again.

3. 9 cDNA synthesis

Synthesis of cDNA from isolated RNA was carried out using Prime Script 1st strand cDNA synthesis kit (Takara, cat. n. 6110A, Japan). RNA was diluted with calculated volume of nuclease free water (2 µg of RNA in a total volume 8 µL). Then 1 µL of 50 µM oligo dT Primer and 1 µL of 10mM dNTP was added. This mixture was kept in thermo cycler for 5 min at 65°C and then it was immediately cooled down on ice. During the cooling time a reaction mixture was prepared by adding 4.5 µL of Rnase-free water, 4 µL of 5× PrimeScript Buffer, 0.5 µL of Rnase Inhibitor (40 U/µL) and 1 µL of PrimeScript Rtase (200 U/µL), and then it was gently mixed with the cold mixture which contained the RNA sample. Final volume of the reaction was 20 µL. The tubes containing reaction mixture were kept in a thermo cycler at 42°C for 60 min and after

that at 70°C for 15 min. To check the integrity of the synthesised cDNA, PCR with *LeGAPDH* primers was performed in the same way as described above. The synthesised cDNA was stored in the freezer at -20°C until performing qPCR to quantify an expression of a gene of interest.

3. 10 Analysis of gene expression

The quantitative real-time polymerase chain reaction (qPCR) was used as a method for studying the relative quantification of transcription levels of two genes. The gene of interest was *BRI1* (*Brassinosteroid-Insensitive1*; accession number: AY179606) which encodes tomato brassinosteroid receptor. As the housekeeping gene *Elongation-factor 1-α* (*EF1α*, accession number: X14449; Bergougnoux *et al*., 2012) was used. For amplification of these genes, primers of following sequences were used:

BRI1-F: 5´-AGT TGA AGG ATG GGA GTG TTG-3´

BRI1-R: 5´-GGT GCT TGA TCT TCC CTA TGG-3´

EF1α-F: 5´-CCC AAG AGG CCA TCA GAC AA-3´

EF1α-R: 5´-CAA CAG GGA CAG TTC CAA TAC CA-3´

The qPCR was performed using the SensiFAST™ SYBR® Lo-ROX Kit (Bioline, UK). The reaction mixture for one reaction was prepared by adding 10 µL of 2× SensiFAST™ SYBR® Lo-ROX Mix, 0.8 µL of 10µM forward primer, the same amount of a reverse primer, 4.4 µL of water and 4 µL of template cDNA (4× diluted in water). The final volume of the reaction was 20 µL. Three technical replicates were performed for each sample. The amplification was carried out in a 2-step cycling program, which consisted of the following steps: denaturation at 95°C for 2 min and then 40 cycles of 95°C for 5 s followed by 15 s at 60°C. A dissociation curve was obtained for each sample. All cycle threshold (Ct) values were normalized against those for the *EF1α* gene and the efficiency of amplification. The results are presented as an expression fold change. It was determined by comparing the differences in the cycle numbers of the samples during linear amplification phase using the ΔΔCt method. The relative quantification was related to the sample taken from the hypocotyls of WT genotype grown in dark condition on the basal medium or medium containing DMSO (for last experiment with BRs treatment). The values represent the mean relative expressions obtained from two, three or four independent experiments.

For the analysis of the data, the program REST 2009 (Pfaffl *et al.*, 2002, REST 2009 Software User Guide, 2009) was used to evaluate whether the differences in *BRI1* expression are statistically significant. The software is using the integrated statistical randomization tests to determine whether a significant difference exists between samples a control. It also takes into account the issues of primers efficiency and reference gene normalization.

4 RESULTS

The purpose of the present study was to contribute to the understanding of the *7B-1* gene function and the interaction between blue light and brassinosteroid signalling. This study is partially based on previous research summed up in the bachelor's thesis (Pilařová, 2014) which reported the altered growth responses to BRs of the *7B-1* mutant hypocotyls compared to WT, and altered level of endogenous brassinosteroids. Therefore, several experiments studying growth response to 24-epiBL and the expression of BR receptor in different conditions were designed and carried out.

4. 1 Growth responses to 24-*epi*brassinolide

Brassinosteroids are biologically active in very low concentrations and they are known to be involved in processes such as photomorphogenesis and cell elongation (Wang *et al.,* 2012). The previous study showed that 24-*epi*BL stimulates the WT epidermal cells elongation under BL only and not the elongation of *7B-1* cells (Pilařová, 2014). Therefore, responses to lower concentrations of 24-*epi*BL (10^{-12} M, 10^{-11} M, 10^{-10} M and 10^{-9} M) were tested in 3 independent experiments to see if there is possible shift in sensitivity to BRs could be observed in *7B-1* mutant. In each biological repeat, 5 plants of each genotype were growing on selected low concentrations of BRs and under each light condition. During the examined period, germination problems occurred and therefore, one of the experiments did not include WT genotype.

As expected, in the dark no essential effect of 24-*epi*BL on hypocotyl elongation was observed in either genotype (Figure 14).

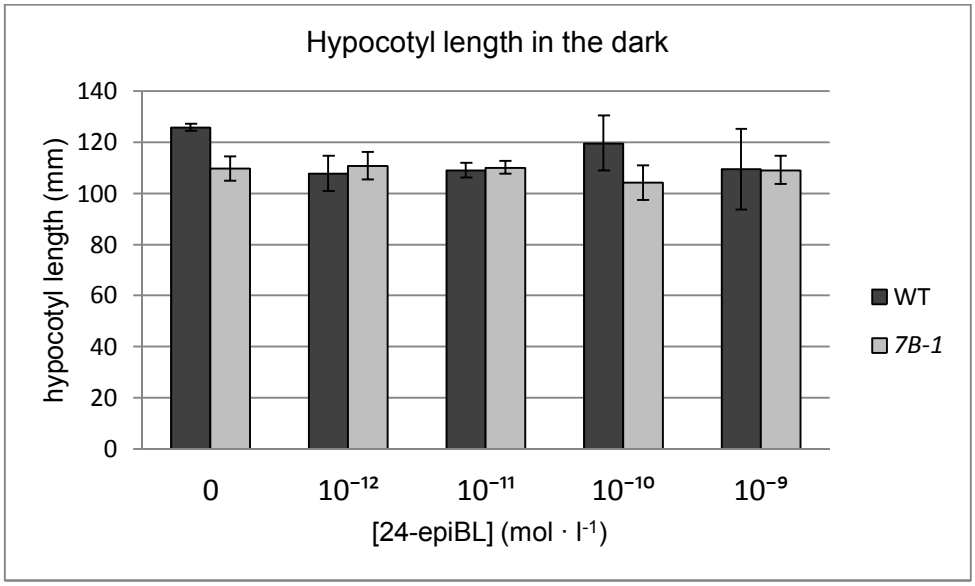


Figure 14: Length of the hypocotyls of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* grown in the dark. The seeds were germinating on the basal MS medium in the dark and then they were transferred on a new basal MS medium (control) or on the medium containing 24-*epi*-brassinolide (in the 10^{-12} M, 10^{-11} M, 10^{-10} M or 10^{-9} M concentration) where they grew for 7 days. Values represent mean ± SE obtained from *n* independent experiments (for WT *n* = 2; for *7B-1 n* = 3).

In RL, the *7B-1* hypocotyls were slightly shorter than those in WT and under these light conditions no effect of exogenous 24-*epi*BL on hypocotyl elongation was observed (Figure 15).

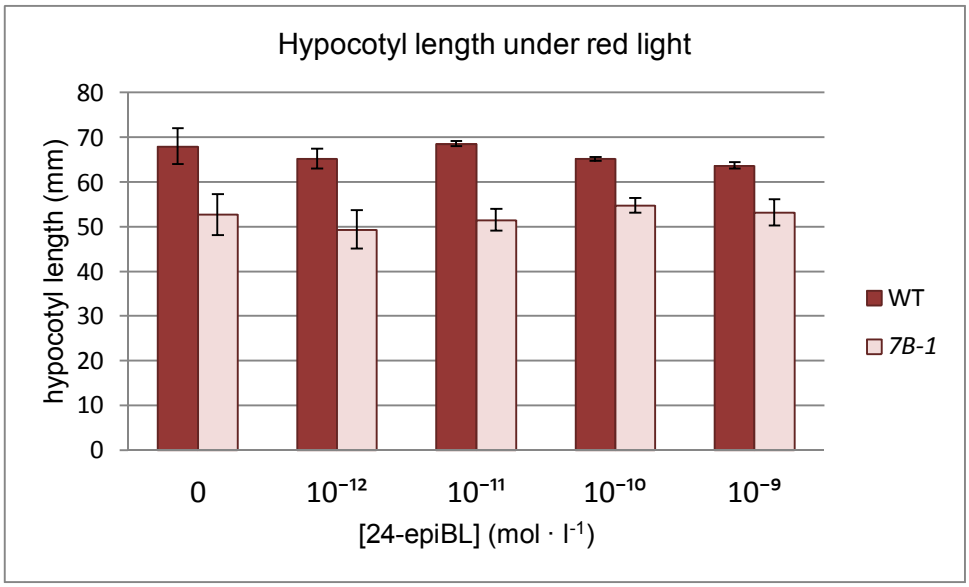


Figure 15: Length of the hypocotyls of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* grown in the red light (RL). The seeds were germinating on the basal MS medium in the dark and then they were transferred on a new basal MS medium (control) or on the medium containing 24-*epi*-brassinolide (in the 10^{-12} M, 10^{-11} M, 10^{-10} M or 10^{-9} M concentration) and under RL where they grew for 7 days. Values represent mean ± SE obtained from *n* independent experiments (for WT *n* = 2; for *7B-1* *n* = 3).

The results of the experiments under BL conditions confirmed the inhibitory effect of BL on hypocotyl elongation in both genotypes, and also the finding that the *7B-1* mutant is less sensitive to BL-induced hypocotyl de-etiolation. However, no effect of 24-*epi*BL on hypocotyl growth was observed in either genotype (Figure 16).

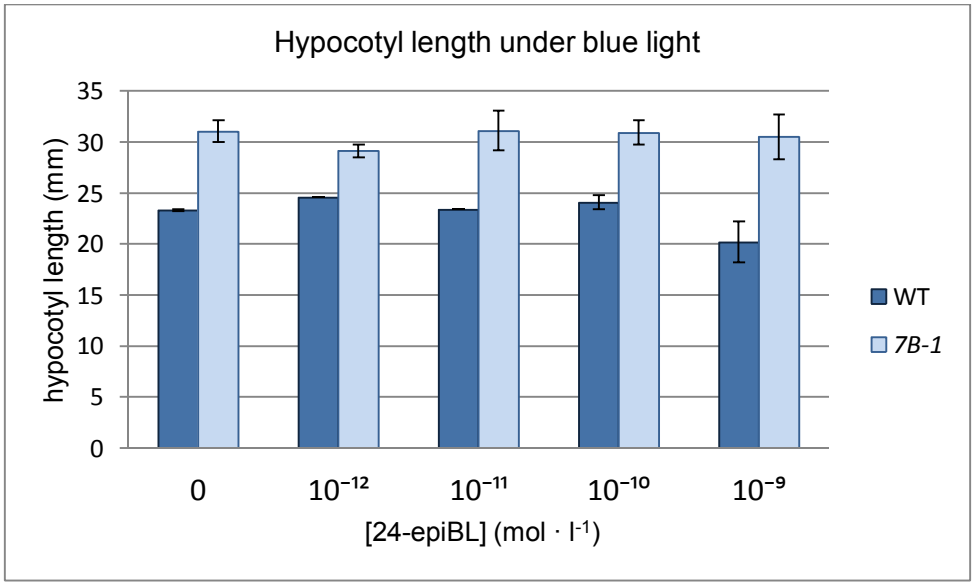


Figure 16: Length of the hypocotyls of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* grown in the blue light (BL). The seeds were germinating on the basal MS medium in the dark and then they were transferred on a new basal MS medium (control) or on the medium containing 24-*epi*-brassinolide (in the 10^{-12} M, 10^{-11} M, 10^{-10} M or 10^{-9} M concentration) and under BL where they grew for 7 days. Values represent the mean ± SE obtained from *n* independent experiments (for WT *n* = 2; for *7B-1 n* = 3).

4. 2 Expression of tomato *BRI1* under blue light

According to the previous study (Pilařová, 2014), BL might reduce the expression of *BRI1* gene for BR receptor and the *7B-1* mutant could express less *BRI1* in the dark. Therefore, the qRT-PCR analysis of hypocotyls grown under different conditions was performed to obtain more accurate data.

4. 2. 1 Seven-day-old hypocotyls

First, the WT and *7B-1* germinating seeds were grown for 7 days in the dark or BL, their mRNA was extracted, cDNA was synthesised and the qRT-PCR was performed to analyse the *BRI1* expression compared to *EF1α* housekeeping gene. Values obtained after the relative quantification were normalised to the expression in WT dark-grown hypocotyls. Four independent experiments were carried out.

The results suggested that there is a trend showing that BL reduces the intensity of *BRI1* expression and that the *7B-1* mutant has lower level of *BRI1* transcript (Figure 17). But none of these differences turned out to be significant in REST 2009.

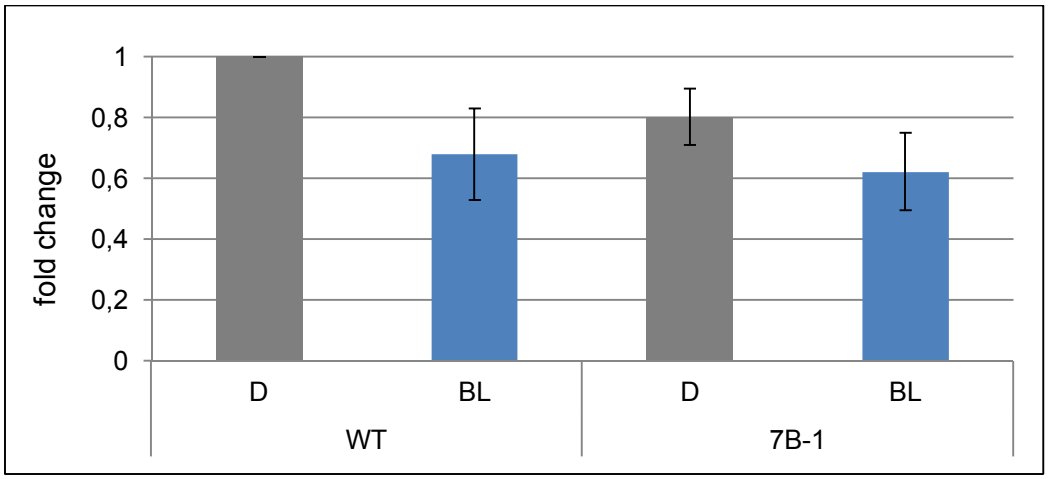


Figure 17: qRT-PCR analysis of the expression of *BRI1* gene in the hypocotyl of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* which germinated in the dark (D) and then they were transferred on new medium and grown in the dark or in blue light (BL) for 7 days. The results represent the mean ± SE of four independent biological repeats. The *EF1α* was used as a housekeeping gene. The relative quantification was made against the expression of *BRI1* in the WT hypocotyls grown in the dark.

4. 2. 2 Three-day-old hypocotyls

The experiments with 7-day-old hypocotyls were followed by another set of experiments with younger plants. The WT and *7B-1* seedlings were grown for 3 days under BL or in the dark before the harvesting and RNA extraction. In the WT 3-day-old hypocotyls, the level of *BRI1* expression was significantly reduced by half ($p < 0.05$) under BL conditions. Although, there was not any change in the *BRI1* expression level between WT and *7B-1* genotypes in BL, the level of *BRI1* transcript in the dark grown *7B-1* mutant was lower than in WT in all three independent biological repeats but the *p* value obtained in REST 2009 was higher than 0.05 (Figure 18).

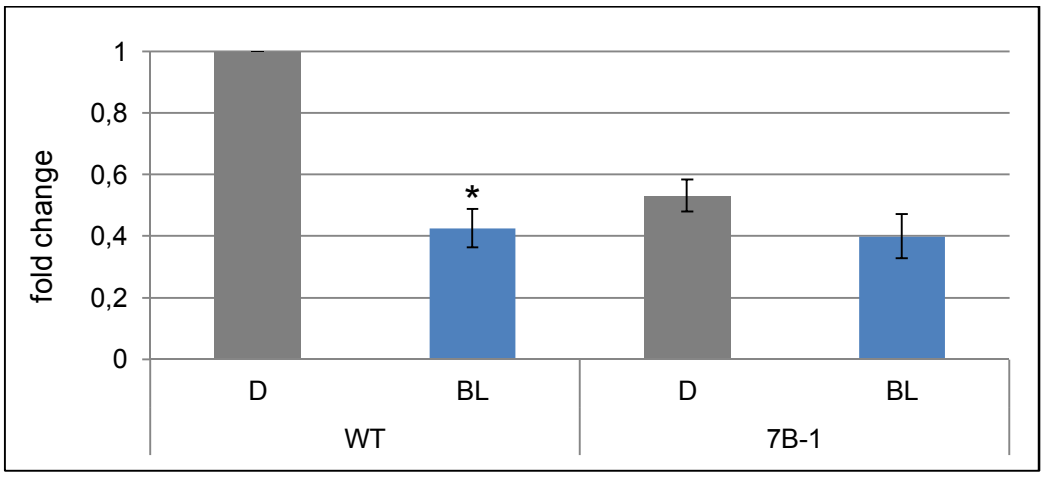


Figure 18: qRT-PCR analysis of the expression of *BRI1* gene in the hypocotyl of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* which germinated in the dark (D) and then they were transferred on new medium and grown in the dark or in blue light (BL) for 3 days. The results represent the mean ± SE of 3 independent biological repeats. The *EF1α* was used as a housekeeping gene. The relative quantification was made against the expression of *BRI1* in the WT hypocotyls grown in the dark. Statistics were calculated in REST 2009 (Pfaffl *et al.*, 2002, REST 2009 Software User Guide, 2009). The asterisk marks significant difference from the WT in D sample ($p < 0.05$).

4. 2. 3 Response to exogenous 24-*epi*brassinolide

Two experiments were designed to find out if the expression of *BRI1* is altered by exogenous 10^{-6} M 24-*epi*BL and whether its possible effect is modulated by BL. Both following experiments were performed only in one biological repeat for time pressure.

First experiment was performed with the 7-day-old WT hypocotyls. The results confirm the reduction of *BRI1* expression by BL but do not show any clear effect of exogenous 24-*epi*BL on the *BRI1* expression (Figure 19).

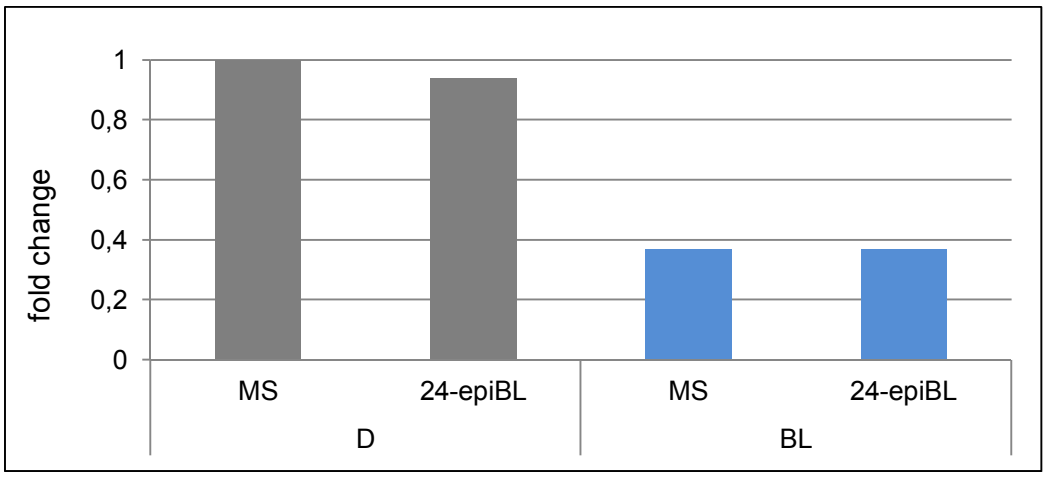


Figure 19: qRT-PCR analysis of the expression of *BRI1* gene in the hypocotyl of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) which germinated in the dark (D) and then they were transferred on basal medium (MS) or containing 10^{-6} M 24-*epi*-brassinolide and grown in the dark or in blue light (BL) for 7 days. The results present one biological repeat. The *EF1α* was used as a housekeeping gene. The relative quantification was made against the expression of *BRI1* in hypocotyls grown on MS medium in the dark.

In the second experiment, the *7B-1* genotype was included to the experiments. In addition, MS medium supplemented with DMSO was used as the control, as DMSO was used as a solvent of the 24-*epi*BL. In the WT, the *BRI1* expression was slightly higher in etiolated hypocotyls treated with 24-*epi*BL than in hypocotyls grown in the presence of DMSO. In contrast, the *BRI1* expression level in *7B-1* was lower in the 24-*epi*BL-treated etiolated hypocotyls than in the DMSO-treated hypocotyls. The results also show that in WT hypocotyls, the expression of *BRI1* is reduced by BL (Figure 20).

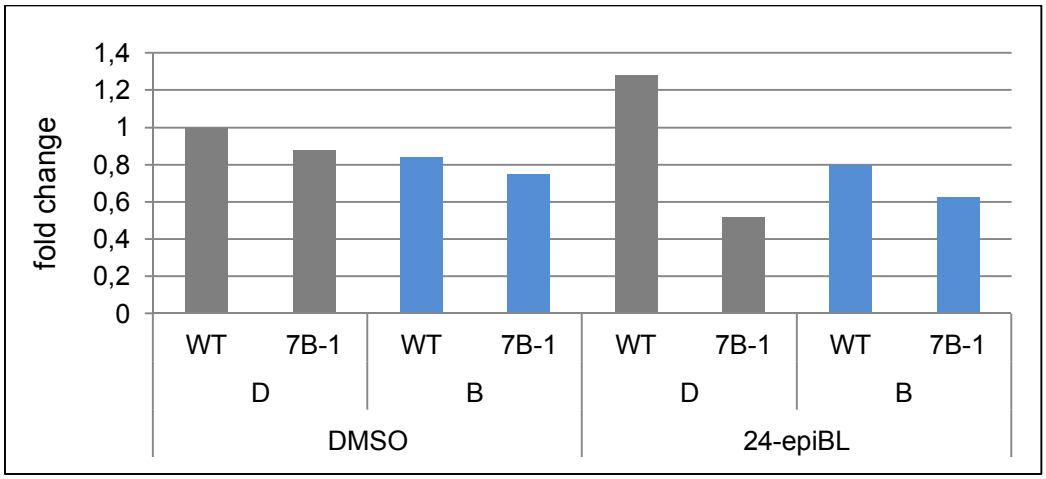


Figure 20: qRT-PCR analysis of the expression of *BRI1* gene in the hypocotyl of *Solanum lycopersicum* L. cv Rutgers (wild-type, WT) and the mutant *7B-1* which germinated on basal MS medium in the dark (D) and then they were transferred on medium containing 10^{-6} M 24-*epi*-brassinolide or DMSO in the same concentration as presented in 24-*epi*BL solution. They grew in the dark or in blue light (BL) for 3 days. The results present one biological repeat. The *EF1α* was used as a housekeeping gene. The relative quantification was made against the expression of *BRI1* in WT hypocotyls grown on medium with DMSO in the dark.

5 DISCUSSION

According to previous studies, the *7B-1* tomato mutant shows reduced hypocotyl (Fellner and Sawhney, 2002) and epidermal cell elongation (Bergougnoux et al., 2012; Pilařová, 2014) under blue light. Also the stomatal opening (Hlavinka et al., 2012) and other phototropin responses (Fellner and Bergougnoux, unpublished data) are affected in the *7B-1* mutant. Therefore, the functional 7B-1 product is likely involved in the phototropin signalling pathway. It is also known that the BL receptor's transcriptional level is not affected by the *7B-1* mutation (Omidvar and Fellner, 2015).

The levels of endogenous phytohormones in the *7B-1* are also altered. The *7B-1* mutant is an ABA over-producer and contains lower levels of auxin, GAs (Fellner *et al*., 2001), ethylene (Fellner *et al*., 2005) and cytokinins (Bergougnoux *et al*., 2012) than the WT. The analysis of endogenous BRs showed that hypocotyls of the *7B-1* mutant have a five times lower level of brassinolide than the WT in the dark but the same level in BL and RL (Pilařová, 2014). Also, *7B-1* growth responses to exogenous 24-*epi*BL were changed compared to the WT. Whereas the WT BL-grown hypocotyl elongation was stimulated by 24-*epi*BL (10^{-9} M, 10^{-8} M, 10^{-7} M and 10^{-6} M), the *7B-1* hypocotyls grown on 24-*epi*BL did not differ from the untreated ones (Figure 4).

The experiments of the present study did not reveal any elongation of the BL-grown hypocotyls after the stimulation with 24-*epi*BL in lower concentrations (10^{-12} M, 10^{-11} M, 10^{-10} M and 10^{-9} M). These concentrations are probably too low to induce hypocotyl elongation. The roots were inhibited by the high levels of 24-*epi*BL in the medium (Pilařová, 2014), whereas the experiments presented in this work suggest that concentrations lower than 10^{-9} M did not inhibit the roots. However, the experiments did not show any root stimulation. As there was a strong variability in the root length (data not showed), for studying the effect of exogenous 24-*epi*BL on the root growth, more experiments have to be carried out to receive reproducible results. Other interesting experiments which could tell us more about the relationship between the hypocotyl elongation under BL and exogenous BRs, could also involve the tomato phototropin mutant isolated by Sharma *et al*. (2014) or cryptochrome mutants *cry1-1* and *cry1-2*, selected by Weller *et al.* (2001).

The experiments presented in this study disprove the hypothesis that the BR-induced elongation of the BL-grown WT hypocotyls is caused by a higher *BRI1* expression level in the BL-grown WT hypocotyls. In fact, the WT hypocotyls expressed significantly less *BRI1* in BL than in the dark. The results also suggest that in the dark, *7B-1* mutant might express less *BRI1* than the WT. No difference was observed in the *BRI1* expression level between the two genotypes under BL.

Previous literature has concentrated on the interaction between light and BR biosynthesis and BR inactivation (e.g. Neff *et al*., 1999; Turk *et al.*, 2003; Turk *et al.*, 2005) and on shade-avoidance controlled by light and BRs (e.g. Keller *et al.*, 2011; Keuskamp *et al.*, 2011; Trupkin *et al.*, 2012), but as far as we know the data about the influence of BL on the first steps of BR signalling has not been reported yet. That is why it would be interesting to test whether the *BRI1* expression level is altered by RL or white light.

In the experiments presented in this study, bigger differences in the expression levels in the younger hypocotyls (3-day-old) then in the 7-day-old ones (Figure 17 and Figure 18) were observed. One explanation for this difference could be that the higher

concentration of the total mRNA in the 3-day-old hypocotyls is present, which could be caused by their more active development and growth. Another explanation might be that the extended BL irradiation in the 7-day-old hypocotyls could cause the inactivation of the BL photoreceptors thus decreasing the sensitivity of the 7-day-old hypocotyls to BL.

The experiments which were focused on the effect of exogenous 24-*epi*BL on the expression of *BRI1* in the hypocotyls did not show any difference between the control and the treated plants. However, the low number of repetitions in these experiments does not allow to state any conclusions about the actual effect of exogenous BRs on *BRI1* expression.

The fact, that the *7B-1* mutant has a lower endogenous BR level and a lower *BRI1* expression and still behaves the same as the WT with respect to the growth responses to exogenous BR in the dark, and at the same time the *7B-1* mutant is not sensitive to exogenous BR in BL, suggests that more than one signalling pathway are involved in hypocotyl growth responses. The working model of the interaction between BL the expression of the BR receptor *BRI1* and the functional product of the *7B-1* gene in plant growth proposed in Pilařová (2014) is presented in Figure 21. The interactions studied in this thesis are highlighted with magenta.

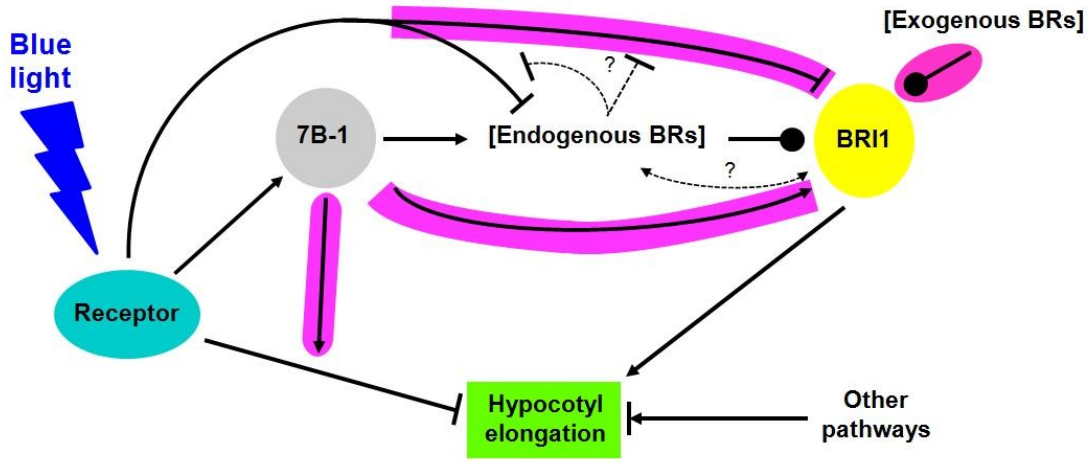


Figure 21: Proposed model of the interactions between blue light, the expression of brassinosteroid receptor *BRI1* and the fully functional product of *7B-1* gene in plant growth. Arrows indicate activation, bar-ended lines inhibition, magenta lines highlight the interactions which were studied in this work. Blue light inhibits hypocotyl elongation and reduces the level of *BRI1* expression as well as the concentration of endogenous BRs. Fully functional product of *7B-1* gene is positively involved in photomorphogenesis. In the dark, it stimulates endogenous BRs level and *BRI1* expression. BRs stimulate hypocotyl elongation and endogenous BRs might stimulate *BRI1* expression or the other way around.

6 CONCLUSIONS AND PERSPECTIVES

The present Master's thesis focused on the interactions of the *7B-1* mutation in the tomato and the signalling of brassinosteroids and blue light. The experiments which were performed examined the growth responses to exogenous 24-*epi*BL of the *7B-1* mutant and the wild type cv. Rutgers in blue light, red light and in the dark. The tested concentrations of 24-*epi*BL did not influence hypocotyl growth.

Another series of experiments were focused on the expression of the BR receptor *BRI1* in the *7B-1* mutant and in the WT under BL and in the dark. The results showed that in etiolated hypocotyls, the *7B-1* mutant has a lower expression level of *BRI1* than WT plants, and these differences were bigger in 3-day-old hypocotyls than in 7-day-old ones. Moreover, BL significantly reduces the expression of *BRI1* in the WT but not in *7B-1* mutant. Finally, the effect of exogenous 24-*epi*BL on the expression level of *BRI1* was tested but none was observed in WT or *7B-1*.

The results obtained in the present thesis, in combination with previous reported data, improve our knowledge of the *7B-1* mutation and its role in the BL and BR pathways. For the first time, the fact that the expression of *BRI1* gene is influenced by BL is reported. This finding shows the possibilities for further investigation of the cross-talk between blue light and brassinosteroid signalling.

7 ABBREVIATIONS

24-*epi*BL	24-*epi*-brassinolide
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
BAK1	BRI1-ASSOCIATED RECEPTOR KINASE
BIN2	BRASSINOSTEROID-INSENSITIVE 2
BKI	BRI1 KINASE INHIBITOR
BR	Brassionsteroid
BRI1	BRASSINOSTEROID INSENSITIVE 1
BRs	Brassinosteroids
BL	Blue light
BSK	BRASSINOSTEROID SIGNALING KINASE
BSU1	BRI1-SUPRESSOR1 BIN2 (BRASSINOSTEROID-INSENSITIVE 2)
CDG1	CONSTITUVITE DIFFERENTIAL GROWTH1
COP1	CONSTITUVE PHOTOMORPHOGENESIS 1
COR	Coronatine
CKs	Cytokinins
cry1	Cryptochrome 1
D	Dark
DMSO	Dimethyl sulfoxide
GAs	Gibberellins
g_s	Stomatal conductance
IAA	Indole-3-acetic acid
iP	Isopentenyladenine
JA	Jasmonic acid
LD	Long day
LRRs	Leucin-rich repeats
PAL	Phenylalanine ammonia-lyase
phot1	Phototropin 1
phot2	Phototropn 2
Phy	Phytochrome
P_N	Net photosynthetic rate
PME	Pectinmethylesterase
PMEI	Pectinmethylesterase inhibitor
RL	Red light
ROC1	ROTAMASE CYCLOPHILIN1
SA	Salicylic acid
SD	Short day
WT	Wild-type
WUE_i	Intrinsic water-use efficiency
XTH	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE

8 REFERENCES

Asahina M, Tamaki Y, Sakamoto T, Shibata K, Nomura T, Yokota T; 2014. Blue light-promoted rice leaf bending and unrolling are due to up-regulated brassinosteroid biosynthesis genes accompanied by accumulation of castasterone. Phytochemistry. **104**: 21–29

Bai MY, Shang JX, Oh E, Fan M, Bai Y, Zentella R, Sun T, Wang ZY; 2012. Brassinosteroid, Gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. Nature Cell Biology. **14**: 810-817

Bajwa VS, Wang XF, Blackburn RK , Goshe MB, Mitra SK, Williams EL, Bishop GJ, Krasnyanski S, Allen G, Huber SC, Clouse, SD; Identification and Functional Analysis of Tomato BRI1 and BAK1 Receptor Kinase Phosphorylation Sites. Plant Physiology. **163**: 30-42

Bergougnoux V, Zalabák D, Jandová M, Novák O, Wiese-Klinkengerg A, Fellner M; 2012. Effect of Blue Light on Endogenous Isopentenyladenine and Endoreduplication during Photomorphogenesis and De-Etiolation of Tomato (*Solanum lycopersicum* L.) Seedlings. PLoS ONE. **7**: e45455

Bergougnoux V, Hlaváčková V, Plotzová R, Novák O, Fellner M; 2009. The *7B-1* mutation in tomato confers a blue light-specific lower sensitivity to coronatine, a toxin produced by *Pseudomonas syringae* pv. *tomato*. Journal of Experimental Botany. **60**: 1219-1230

Borthwick HA, Hendricks SB, Parker MW, Toole EH, Toole VK; 1952. A Reversible Photoreaction Controlling Seed Germination. Proceedings of the National Academy of Sciences of the USA. **38** (8): 662–666

Chen BY; 1997. PCR cloning of multiple glyceraldehyde 3-phosphate dehydrogenase cDNAs from tomato fruit. Unpublished; Gene bank, accession U97257

Choi D, Choi J, Kang B, Lee S, Cho Y, Hwang I, Hwang D. 2014. iNID: An Analytical framework for *I*dentifying *N*etwork Models for *I*nterplays among *D*evelopmental Signaling in *Arabidopsis.* Molecular Plant. **7** (5): 792–813

Christie JM, Reymond P, Powell GK, Bemasconi P, Raibekas AA, Liscum E, Briggs WR; 1998. Arabidopsis NPH1: a flavoprotein with the properties of a photoreceptor for phototropism. Science. **282**: 1698-1701

Clouse SD; 2011. Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks regulating plant development. Plant Cell **23**: 1219–1230

Clouse SD, Langford M, McMorris TC; 1996. A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiology. **111**: 671–78

Clouse SD, Sasse JM; 1998. Brassinosteroids: Essential Regulators of Plant Growth and Development. Annual Review of Plant Physiology and Development. **49**: 427-451

Daie J, Campbell WF; 1981. Response of Tomato Plants to Stressful Temperatures. Plant Physiology. **67**: 26-29

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 stresses. Theoretical 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 abscicic 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

Fridman Y, Savaldi-Goldstein S; 2013. Brassinosteroids in growth control: How, when and where. Plant Science. **209**: 24-31

Goldbach H, Michael G; 1976. Abscisic Acid Content of Barley Grains During Ripening as Affected by Temperature and Variety. Crop Science. **16**: 797-799

Grove MD, Spencer GF, Rohwedder WK; 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature. **281**: 216-217

He JX, Gendron JM, Sun Y, Gampala SS, Gendron N; 2005. BZR1 is a transcriptional repressorwith dual roles in brassinosteroid homeostasis and growth responses. Science. **307**: 1634–38

He JX, Gendron JM, Yang Y, Li J,Wang ZY; 2002. The GSK3-like kinase BIN2 phosphorylates anddestabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in *Arabidopsis*. Proceedings of the National Academy of Sciences of the USA. **99**: 10185–90

Hlavinka J, Nauš J, Fellner M; 2013. Spontaneous mutation *7B-1* in tomato impairs light-induced stomatal opening. Plant Science. **209**: 75-80

Hothorn M, Belkhadir Y, Dreux M, Dabi T, Noel JP; 2011. Structural basis of steroid hormone perception by the receptor kinase BRI1. Nature. **474**: 467-471

Ježilová E, Fellner M, Bergougnoux V, Špundová M; 2012. Is the rate of photosynthesis under blue light altered in the *7B-1* tomato mutant? Photosynthetica. **50**: 477-480

Jones R, Mansfield TA; 1970. Suppression of stomatal opening in leaves, treated with ABA. Journal of Experimental Botany. **21**: 714-718

Kagawa T, Sakai T, Suetsugu N, Oikawa K, Ishiguro S, Kato T, Tabata S, Okada K, Wada M; 2001. Arabidopsis NPL1: a phototropin homolog controlling the chloroplast high-light avoidance response. Science. **291**: 2138-2141

Keller MM, Jaillais Y, Pedmale UV, Moreno, JE, Chory, J, Ballare, CL; 2011. Cryptochrome 1 and phytochrome B control shade-avoidance responses in Arabidopsis via partially independent hormonal cascades. Plant Journal. **67** (2): 195–207

Keuskamp DH, Sasidharan R, Vos I, Peeters AJM, Voesenek LACJ, Pierik R; 2011. Blue-light mediated shade avoidance requires combined auxin and brassinosteroid action in *Arabidopsis* seedlings. The Plant Journal. **67**: 208–217

Kim TW, Guan S, Burlingame AL, Wang ZY; 2011. The CDG1 kinase mediates brassinosteroid signaltransduction from BRI1 receptor kinase to BSU1 phosphatase and GSK3-like kinase BIN2. Molecular Cell. **43**: 561–71

Kim TW, Guan S, Sun Y, Deng Z, Tang W; 2009. Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. Nature Cell Biology. **11**: 1254–60

Kim B, Jeong YJ, Corvalan C, Fujioka S, Cho S, Park T, Choe S; 2014. Darkness and gulliver2/phyB mutation decrease the abundance of phosphorylated BZR1 to activate brassinosteroid signaling in Arabidopsis. The Plant Journal. **77**: 737-747

Kinoshita T, Cano-Delgado A, Seto H, Hiranuma S, Fujioka S; 2005. Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. Nature. **433**: 167–71

Kircher S, Kozma-bognar L, Kim L, Adam E, Harter K, Schafer E, Nagy F; 1999. Lightquality-dependent nuclear import of the plant photoreceptors phytochrome A and B. Plant Cell. **11**: 1445-1456

Kutschera U, Wang YZ; 2012. Brassinosteroid action in flowering plants: a Darwinian perspective. Journal of Experimental Botany. **63** (10): 3511–3522

Kriedemann PE, Loveys BR, Fuuler GL, Leopold AC; 1972. Abscisic acid and the stomatal regulation. Plant Physiology. **49**: 842-847

Li J, Chory J; 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell. **90**: 929–938

Li H, Zhang J, Vierstra RF, Li H; 2010. Quaternary organization of a phytochrome dimer as revealed by cryoelectron microscopy. Proceedings of the National Academy of Sciences of the USA. **107** (24): 10872-10877

Li J, Nagpal P, Vitart V, McMorris TC, Chory J; 1996. A role for brassinosteroids in light-dependent development of *Arabidopsis*. Science. **272**: 398–401

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC; 2002. BAK1, an *Arabidopsis* LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. Cell **110**: 213–22

Luo XM, LinWH,Zhu S, Zhu JY, Sun Y; 2010. Integration of light- and brassinosteroid-signaling pathways by a GATA transcription factor in *Arabidopsis*. Dev. Cell. **19**: 872–83

Mäntylä E, Lang V, Palva ET; 1995. Role of abscisic acid in drought-induced freezing tolerance, cold acclimatization, and accumulation of LTI78 and RAB18 proteins in Arabidopsis. Plant Physiology. **107**: 141–148

Mas P, Kim WY, Somers DE, Kay SA; 2003. Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. Nature. **426**: 567–570

Murashige T, Skoog F; 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum. **15**: 473–497

Nam KH, Li J; 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell. **110**: 203–12

Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Takatsuo S, Yoshida S, Chory J; 1999. BAS1: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proceedings of National Academy of Science of the USA. **96**: 15316-15323

Ni M, Tepperman JM, Quail PH; 1998. PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix-loophelix protein. Cell. **95**: 657–667

Noguchi T, Fujioka S, Choe S, Takatsuto S, Tax FE, Yoshida S, Feldmann KA; 2000. Biosynthetic pathways of brassinolide in Arabidopsis. Plant Physiology. **124** (1): 201-209

Oh E, Zhu JY, Wang ZY; 2012. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. Nature Cell Biology. **14**: 802-809

Omidvar V, Mohorianu I, Dalmay T, Fellner M; 2015a. Identification of miRNAs with potential roles in regulation of anther development and male-sterility in *7B-1* male-sterile tomato mutant. BMC Genomics. **16**: 878- 893

Omidvar V, Mohorianu I, Dalmay T, Fellner M; 2015b. MicroRNA Regulation of Abiotic Stress Response in *7B-1* Male-Sterile Tomato Mutant. The Plant Genome. **8** (3)

Parihar P, Singh R, Singh S, Tripathi DK, Chauhan DK, Singh VP, Prasad SM; 2016. Photoreceptors mapping from past history till date. Journal of Photochemistry & Photobiology, B: Biology. **162**: 223- 231

Pfaffl MW, Horgan GW, Dempfle L; 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research. **30** (9)

Pilařová L; 2014. Růstové reakce mutanta *7B-1* k barssinosteroidům v závislosti na světelných podmínkách. Bachelor's Thesis. Palacký University Olomouc. Faculty of Science.

REST 2009 Software User Guide; 2009. *http://rest-2009.gene-quantification.info/* (online). Available on: http://rest-2009.gene-quantification.info/. acceded on 10. 07. 2016.

Sawhney VK; 1997. Genetic male sterility. In: Shivanna KR, Sawhney VK, Pollen biotechnology for crop production and improvement. Cambridge, UK: 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 & Biotechnology. **79**: 138-141

Szekeres M, Nemeth K, Koncz-Kalman Z, Mathur J, Kauschmann A; 1996. Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in *Arabidopsis*. Cell. **85**: 171–182

Seo HS, Watanabe E, Tokutomi S, Nagatanni A, Chua NH; 2004. Photoreceptor ubiquitination by COP1 E3 ligase desensitizes phytochrome A signaling. Genes & Development. **18**: 617-622

Sharma S, Kharshiing E, Srinivas A, Zikihara K, Tokutomi S, Nagatani A, Fukayama H, Bodanapu R, Behera RK, Sreelakshmi Z, Sharma R; 2014. A Dominant Mutation in the Light-Oxygen and Voltage2 Domain Vicinity Impairs Phototropin1 Signaling in Tomato. Plant Physiology. **164**: 2030–2044

Sharrock RK, Quail PH; 1989. Novel phytochrome sequences in Arabidopsis thaliana: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes & Development. **3**: 1745-1757

She J, HanZ, KimTW, Wang J, Cheng W; 2011. Structural insight into brassinosteroid perception by BRI1. Nature. **474**: 472–76

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

Sheoran IS, Ross ARS, Olson DJH, Sawhney VK; 2009. Differential expression of proteins in the wild type and 7B-1 male-sterile mutant anthers of tomato (*Solanum lycopersicum)*. A proteomic analysis. Journal of Proteomics. **71**: 624-636

Spollen WG, Lenoble ME, Samuels TD, Bernstein N, Sharp RE; 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. Plant Physiology. **122**: 967–976.

Sun Y, Fan XY, Cao DM, Tang W, He K; 2010. Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in *Arabidopsis*. Developmental Cell. **19**: 765–77

Symons GM, Schultz L, Kerckhoffs LHJ; 2002. Uncoupling brassinosteroid levels and de-etiolation in pea. Physiologia Plantarum. **115**: 311-319

Symons GM, Smith JJ, Nomura T, Davies NW, Yokota T, Reid JB; 2008. The hormonal regulation of de-etiolation. Planta. **227**: 1115–25

Taiz L, Zeiger E; 2010. Plant Physiology, 5th ed., Sumderland, Mass.: Sinauer Associates

Tang W, Kim TW, Oses-Prieto JA, Sun Y,Deng Z; 2008. BSKs mediate signal transduction from the receptor kinase BRI1 in *Arabidopsis*. Science. **321**: 557–560

Thompson MJ, Mandava NB, Meudt WJ. Lusby WR. Spaulding DW; 1981. Synthesis and biological activity of brassinolide and its 22β,23β-isomer: Novel plant growth-promoting steroids. Steroids. **38**: 567-580

Traas J, Hülskamp M, Gendreau E, Höfte H; 1998. Endoreduplication and development: rule without dividing?. Current Opinion in Plant Biology **1**: 198-503

Trupkin SA, Mora-Garcia S, Casel JJ; 2012. The cyclophilin ROC1 links phytochrome and cryptochrome to brassinosteroid sensitivity. Plant Journal. **71**: 712-723

Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, Denzel MA, Torres QI, Neff MM; 2003. CYP72B1 inactivates brassinosteroid hormones: An intersection between photomorphogenesis and plant steroid signal transduction. Plant Physiology. 133: 1643-1653

Turk EM, Fujioka S, Seto H, Shimada Y, Takatsuto S, Yoshida S, Wang H, Torres Q, Ward JM, Murthy G, Zhang J, Walker JC, Neff MM; 2005. *BAS1* and *SOB7* act redundantly to modulate Arabidopsis photomorphogenesis via unique brassinosteroid inactivation mechanisms. Plant Journal. **42**: 23-34

Vriet C, Russinova E; Reuzeau C; 2013. From squalene to brassinolide: The steroid metabolic and signaling pathways across the plant kingdom. Molecular Plant. **6**: 1738-1757

Wada K, Marumo S, Ikekawa N; 1981. Brassinolide and homobrassinolide promotion of lamina inclination of rice seedlings. Plant and Cell Physiology. **22**: 323-325

Wang X, Chory J; 2006. Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. Science. **313**: 1118–1122

Wang X, Kota U, He K, Blackburn K, Li J; 2008. Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Developmental Cell. **15**: 220–235

Wang X, Li X, Meisenhelder J, Hunter T, Yoshida S; 2005. Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. Developmental Cell. **8**: 855–865

Wang ZY, Bai MY, Oh E, Zhu JY; 2012. Brassinosteroid Signaling Network and Regulation of Photomorphogenesis. Annual Review of Genetics. **46**: 701-724.

Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J; 2001. BRI1 is a critical component of a plasmamembrane receptor for plant steroids. Nature. **410**: 380–383

Weller JL, Perrotta G, Schreuder ME, van Tuinen A, Koornneef M, Giuliano G, Kendrick RE; 2001. Genetic dissection of blue-light sensing in tomato using mutants deficient in cryptochrome 1 and phytochromes A, B1 and B2. Plant Journal. **25:** 427–440

Whippo CW, Hangerter RP; 2005. A Brassinosteroid-Hypersensitive Mutant of BAK1 Indicates That a Convergence of Photomorphogenic and Hormonal Signaling Modulates Phototropism. Plant Physiology. **139**: 448-457

Yang HQ, Wu YJ, Tang RH, Liu DM, Liu Y, Cashmore AR; 2000. The C termini of Arabidopsis cryptochromes mediate a constitutive light response. Cell. **103**: 815-827.

Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J; 2005. A New Class of Transcription Factors Mediates Brassinosteroid-Regulated Gene Expression in *Arabidopsis*. Cell. **120**: 249-259

Yin YH, Wang ZY, Mora-Garcia S; 2002. BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. Cell. **109**: 181-191

Yokotani N, Nakano R, Imanishi S, Nagata M, Inaba A, Kubo Y; 2009. Ripening-associated ethylene biosynthesis in tomato fruit is autocatalycally and developmentally regulated. Journal of Experimental Botany. **60** (12): 3433-3442

Zhang X, Takemiya A, Kinoshita T, Shimazaki K; 2007. Nitric oxide inhibits blue light-specific stomatal opening via abscisic acid signalling pathways in *Vicia* guard cells. Plant and Cell Physiology. **48**: 715-723

Zhang X, Zang R, Li C; 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. Plant Science. **166**: 791-797