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Diploma Thesis

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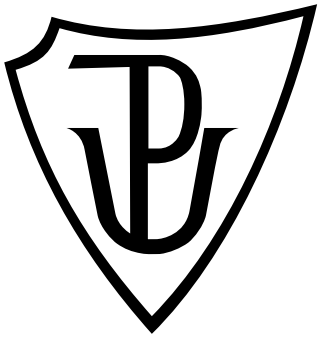

Role of E3 ubiquitin ligase COP1 in boron-regulated hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh.

Diploma thesis

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Úloha E3 ubiquitin ligázy COP1 v bórem regulovaném růstu hypokotylu u *Arabidopsis thaliana* (L.) Heynh.

Diplomová práce

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Olomouc 2013 **Vedoucí práce: Doc. RNDr. Martin Fellner, Ph.D**

DECLARATION

I hereby declare that I elaborated this diploma thesis independently under supervision of Associate Prof. Martin Fellner, Ph.D. with the only use of literature cited in the References.

In Olomouc 5th April 2013 ……..……

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SUMMARY

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CONTENT

1 INTRODUCTION

Boron significantly influences the yield and quality of many crops. Due to the fact that boron deficiency is more extensive than lack of any other plant micronutrient, it is important to closer characterize the boron signaling pathway. Previous results of Kocábek *et al.* (2009) showed that boron and light signaling pathway interact during plant growth and development. Stočes *et al.* (2012) reported that boron and blue light reduced the responsiveness of *Arabidopsis* hypocotyls to exogenous auxin. On the basis of these results, we set a goal to examine which elements of light-signaling pathways are involved in boron-induced elongation of hypocotyl in *Arabidopsis thaliana* and how boron-induced hypocotyl growth is associated with transcription of *BOR1* and *NIP5;1* gene with respect to light and boron concentration. Furthermore, we wished to determine whether these selected photomorphogenesis-related compounds are involved in boron effects on auxin-induced inhibition of hypocotyl elongation. We focused on key element of photomorphogenesis, COP1, on photomorphogenesis-promoting factors LAF1 and HFR1, and blue-light photoreceptor ZTL1.

1 BACKGROUND

1.1 *ARABIDOPSIS THALIANA* – THE MODEL PLANT

Arabidopsis thaliana (L.) Heynh., member of the *Brassicaceae* family, is native to Europe and central Asia but recently is expanded at many places all over the world (Al-Shehbaz and O'Kane, 2002). In the first instance, biologists have turned their attention to this little weed as a model system in genetics (Redei, 1992). Currently, it has become the model plant for a wide range of studies such as physiology, biochemistry or molecular and developmental biology (Pigliucci, 2002).

Special characters make the species ideal for several fields of studies. In the case of the diploid and self-fertile *Arabidopsis* is applied mainly small size of the genome (146 Mbp), which is distributed in five chromosomes (2n=10). Whole genome of this versatile model plant was completed in 2000 and we have in hand the sequences of the approximately 25,500 genes (The Arabidopsis Genome Initiative, 2000). Another *Arabidopsis* advantage is entire life cycle, which can be completed in six to eight weeks. At the end of this time every plant produces several hundred siliques with thousands of seeds. The plants usually reach 25 – 30 cm in height that is economically important character. *Arabidopsis* can be easily grown in a relatively small space in the greenhouse or indoor growth chamber. Very significant is also the fact that mutation can be easily generated by treating seeds with chemicals (Meinke *et al*., 1998) or by transformation using T-DNA insertion method (Krysan *et al*., 1999).

In other words, the model plant research is a necessity for us. In the ever-increasing human population, an increase food production is needed to feed as many people as possible from the smallest area. That's why it is required to understand the developmental and growth processes in plants. Moreover, an extra study of each organism in the whole range is not needed. Therefore, several plants were selected, mostly very simple organisms, which in a relatively short time allow to clarify basic principles of biology with direct relevance to a variety of species, including human. It was observed that 70% human genes involved in cancer have *Arabidopsis* orthologs. Therefore, *Arabidopsis thaliana* is the key model for the biology research of all multicellular eukaryotes (Jones *et al.*, 2008).

1.2 LIGHT AND PHOTORECEPTORS

It is not surprising that light is one of the limiting factor for the plants. It is a source of energy as well as a source of information. The plants respond very sensitively to light, in particular to its intensity, color and direction. From the whole electromagnetic spectrum of the light, only the photosynthetically active radiation (PAR; 400-700 nm wavelengths) is utilized. In addition, the plants have evolved sophisticated system of photoreceptors, which absorb light of different wavelengths and trigger a cascade of multiple signaling pathways resulting in activation or repression of various genes (Neff *et al*., 2000; Aphalo *et al*., 2006).

Especially blue and red portion of the light spectrum is useful to plants. The most inspected are phytochromes (Phys), photoreceptors of red and far-red light. We can recognize two classes of phytochromes. The first class is called Type I., which involves photo-labile photoreceptors and controls Very-Low-Fluence-Responses (VLFR) and far-red High-Irradiance Responses (FR-HIR). Only PhyA assigns to this group. The second class is named Type II., involving relatively light stabile photoreceptors, which control Low Fluence Responses (LFR) and red light High Irradiance Responses (R-HIR). It includes PhyB, PhyC, PhyD and PhyE (Kevei *et al*., 2007, Debrieux and Fankhauser, 2010). Moreover, each of the phytochrome exists in two forms, which differ in absorption of dissimilar wavelengths. While biologically inactive form P_r absorbs red, active P_{fr} form absorbs mainly near far-red region of the light spectrum. Phytochrome is synthesized in the dark *de novo* as P_r. The reversible transformation P_r on P_{fr} occurs due to the light absorption. When P_{fr} absorbs far-red light, it is converted back to P_r form (Kevei *et al*., 2007; Franklin and Qualin, 2010). Upon the activation by light, phytochrome moves from the cytoplasm to nucleus where it triggers signaling pathway leading to series of well-documented responses resulting for example to seedling de-etiolization, seed germination, timing of flowering, leaf and primordia expansion (Briggs and Olney, 2001; Franklin and Qualin, 2010).

The second group of important photoreceptors include receptors of blue light, which can be divided into three groups - cryptochromes (CRY1, CRY2, CRY3), phototropins (PHOT1, PHOT2) and LOV/F-box/Kelch domain proteins (ZTL, FKF and LKP2). The latter are linked primarily with circadian rhythms and flowering induction (Yu *et al*., 2010). Cryptochromes are photolyase-like flavoproteins activated via

phosphorylation in blue light. CRY1 and CRY2 fulfill the function primarily in the nucleus, while CRY3 is active in the chloroplast and mitochondria. Cryptochromes are attributed with a number of physiological processes such as blue-light regulated de-etiolation, control of flowering, stomata opening, root growth, apical dominance, programmed cell death, shade avoidance. The plant height, fruit size, high-light responses, osmotic stress responses and responses to bacterial and viral pathogen are also affected by cryptochromes (reviewed by Liu *et al*., 2011). It was also shown that CRY3 acts as a single stranded DNA repairing enzyme (Selby and Sancar, 2006). The last one, phototropins belong to the blue light-activated serine/threonine kinases. They are involved in phototropism, light-induced stomata opening and chloroplast movements in response to changes in light intensity (Christie, 2007). Furthermore, chlorophyll as well as phytochromes can also absorb blue light and then can mediate the blue-light responses.

1.3 PHOTOMORPHOGENESIS

Photosynthesis, phototropism and photoperiodism are not the only processes impacted by the light. Likewise, the absence or presence of light affects major developmental changes in plant form and structure from seed germination, stem elongation through leaf expansion or flowering (Aphallo *et al*., 2006). With respect to this, two contrasting programs of plant development were described. The seedlings kept in the presence of light adopt the photomophogenesis program. These seedlings are characterized by short hypocotyl, photosynthetically active and highly differentiated cotyledons and the development of green chloroplasts (Fig. 1A) (Jiao *et al*., 2007). In opposite, the seedlings grown in darkness undergo skotomorphogenesis program and display the long hypocotyl, closed cotyledons and apical hook and their proplastids are changed into etioplasts (Fig. 1B) (Wang *et al*., 2002). Genetic, physiological and molecular studies have identified both positive and negative regulators of these developmental mechanisms. COP1 is one of the most intensively studied negative regulators of photomorphogenesis (Nemhauser and Chory, 2002).

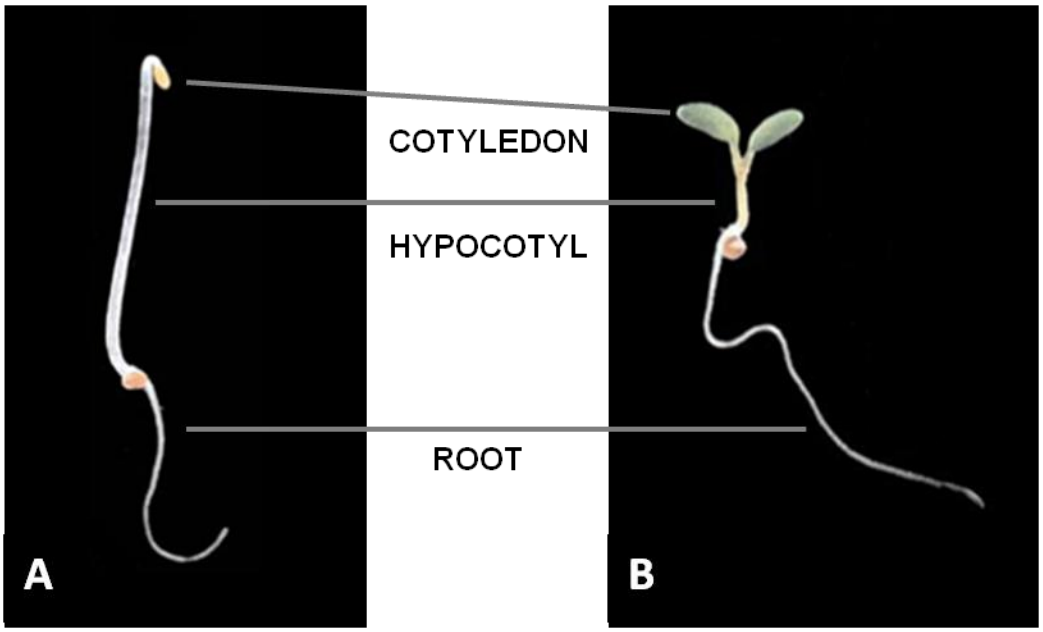


Fig. 1 *- The contrasting phenotypes of dark- vs. light-grown Arabidopsis seedlings (according to Nemhauser and Chory, 2002)*

1.4 COP1 SIGNALING

CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) is a member of the E3-ubiquitin ligases of COP/DET/FUSCA family that was shown to impact negatively the plant photomorphogenesis. The absence of COP1 caused the seedling lethality. However, weak but viable *cop1* mutants generated by the pleiotropic effect indicate the involvement of COP1 in the number of biological processes (Smirnova *at al*., 2011).

Cell cycle, especially regulation of cell proliferation in light-controlled *Arabidopsis* meristem is mediated via COP1 that influences the stability of transcription factors E2FB and E2FC (Lopez-Jues *et al*., 2008). COP1 acts also as a positive regulator of lateral root formation (Ang *et al*., 1998) and mediates the *Arabidopsis* resistance to turnip crinkle virus (TCV) via proteasomic degradation of HRT (Hypersensitive response to TCV) in the dark, while in light, the decreased amount of COP1 is sufficient to ensure HRT resistance (Jeong *et al*., 2010). COP1 contributes in the closed stomata in the darkness (Mao *et al.*, 2005). The mechanism is still unknown but Wang *et al*. (2010) speculated that COP1 can affect the MYB60, the transcription factor required for the stomata opening. UV-B responses such as inhibition of hypocotyl elongation, flavonoid accumulation or changes in gene expression are affected by COP1 (Jenkins *et al*., 2009). The flowering time is

suppressed due to the COP1-mediated proteolysis of CONSTANS (CO) in the dark, while in light, CRY1 participates in the inhibition of COP1 activity and CO can activate expression of *FLOWERING LOCUS T* (*FT*) and resulting in flowering (Liu *et al*., 2008). COP1 is also involved in the light-regulated hormone signaling pathways. In blue light, cytokinins are the repressors of COP1-regulated HY5 degradation, which results in the anthocyanin accumulation (Vandenbubussche *et al*., 2007). Seed germination and seedling development is enhanced by COP1 via HY5, which is required for ABA-inducible gene expression (Chen *et al*., 2008). The deficit of brassinosteroids represses the transcription of *GATA2*, which blocked the process of photomorphogenesis induced by light (Luo *et al*., 2010). COP1 is involved in shoot to root auxin transport via auxin efflux carrier PIN1, and also distribution of PIN proteins within the cell is affected by COP1 (Sassi *et al.,* 2012).

The function of COP1 is modulated by the nucleoplasmic translocation (Fig. 2) in the hypocotyls and cotyledons but not in root cell, where COP1 is constantly present in the nucleus. In dark-grown hypocotyl or cotyledons, COP1 is localized in the nucleus in complex with COP9 signalosome (consists of CSN1 to CSN9 proteins), and DDT protein group (consists of COP10, DDB1, DET1). Absence of COP1 protein in the nucleus of etiolated hypocotyls in mutants of COP9 signalosome suggested that interaction between COP1 and proteins of this complex affected the nucleus-cytosol COP1 movements (Chamovitz *et al*., 1996). But they also suggested that the interaction is not direct, probably it is mediated through some intermediates or specific protein modification. Wang *et al.* (2009) demonstrated that only CSN1 but not CSN4 or CSN5 are required for the COP1 nuclear localization. Nuclear localization signal (NLS), a part of COP1 protein coordinated the presence of COP1 in the nucleus, is essential for COP1 translocation within the cell (Stacey *et al*., 1999, 2000). Matsui *et al.* (2005) described COP1 INTERACTIVE PROTEIN 1 (CIP1), which also participates in the proper COP1 nuclear localization.

Thus, COP1 is the most active in darkness in the form of tetrameric complex consisting of two COP1 proteins and a homo- or hetero-dimer of SPA proteins (Saijo *et al*., 2003). This complex participated on the degradation of transcription factors and photoreceptors associated with the specific light responses. HY5 (Ang *et al*., 1998, Osterlund *et al*., 2000), LAF1 (Seo *et al*., 2003) and HFR1 (Jang *et al*., 2005) are the most intense studied photomorphogenesis-promoting transcription factors,

which are pointed by E3-ubiquitin ligase COP1 to the proteolytic degradation via 26S proteasome under one or several wavelengths (Fig. 3).

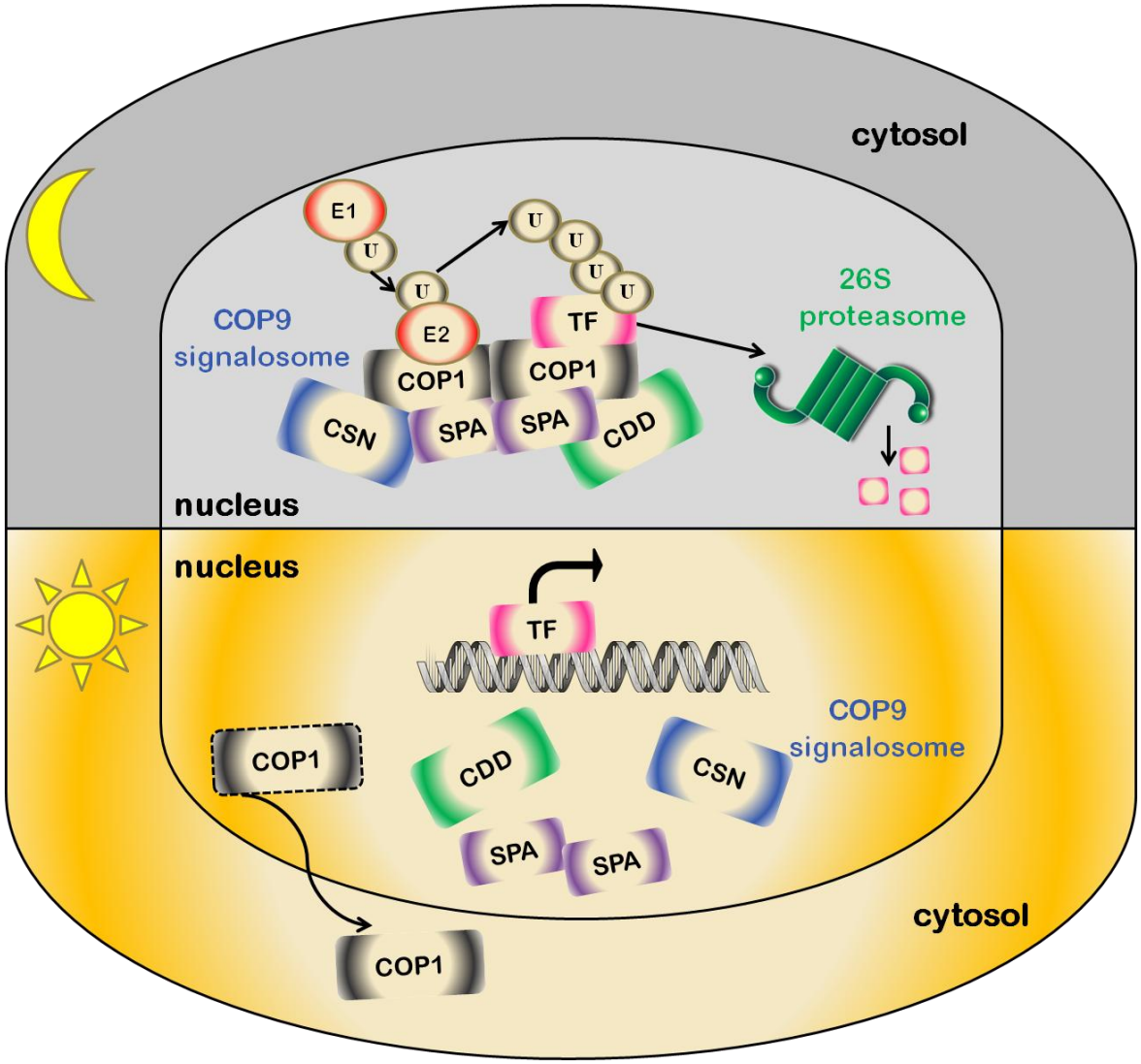


Fig. 2 *– A simplified model of light signaling in plant cell during photomorphogenesis. In darkness in the nucleus of plant cell, COP1/SPA, CSN and CDD complexes co-operate to promote the degradation of photomorphogenesis-promoting transcription factors such as HY5, LAF1, HFR1 with the involvement of 26S proteasome (according to Waters and Langdale, 2009). The light exposure triggers the dissociation the COP/SPA/CDD/CSN complex, COP1 translocation to the cytosol and thus allows the increase in the levels of the photomorphogenesis-promoting transcription factors.*

Long HYpocotyl 5 (HY5) is a bZIP transcription factor with the role in inhibition of hypocotyl elongation, greening, anthocyanin accumulation and lateral root development (Cluis *et al*., 2004). The association between HY5 and G-box promoters is essential for the optimal light activation of these promoters and corresponding genes and then for the proper plant development (Chattopadhyay *et al*., 1998).

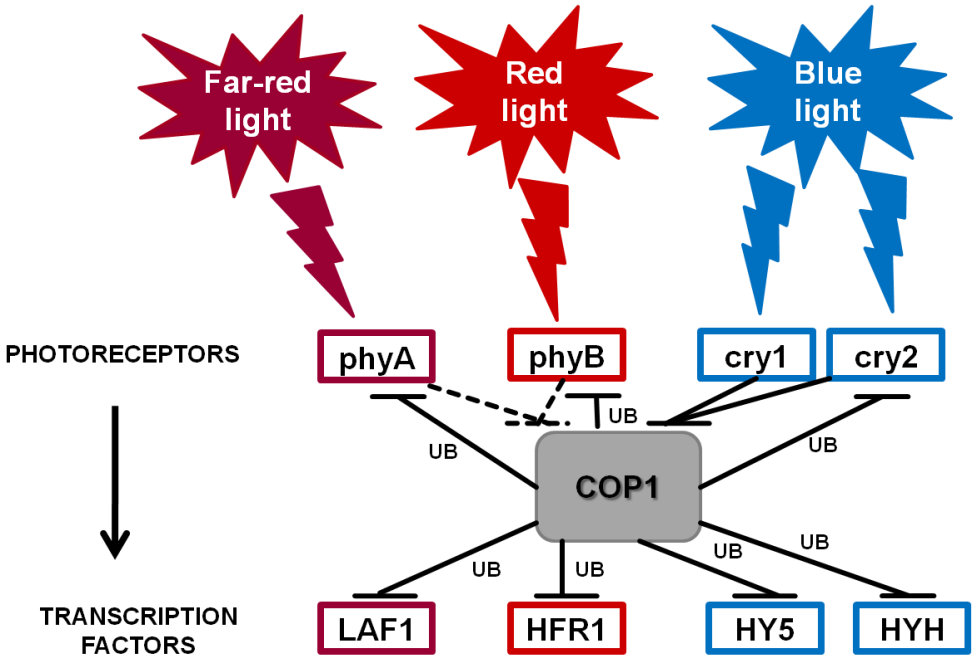


Fig. 3 *- COP1 acts as a central switch in light control of A. thaliana (according to Chunling et al., 2005)*

Long After Far-red light 1 (LAF1) is a R2R3-MYB transcription factor with the positive regulatory function on complete phyA signaling that regulates mainly the inhibition of hypocotyl growth and *CAB*, *PETE*, *XTR7* and *CHS* gene expression (Ballesteros *et al*., 2001). LAF1 regulates the auxin signaling by controlling the expression of auxin response-related genes or auxin efflux carries (Park *et al.,* 2011). Long hypocotyl in far-red 1 (HFR1) is a transcription factor with a bHLH domain. HFR1 is involved in phyA-mediated far red light responses such as hypocotyl growth inhibition, modulation of gravitropic hypocotyl growth or induction of CAB expression. HFR1 also participates in CRY1-dependent blue light signaling, such as the regulation of seedling de-etiolation, and negatively influences shade avoidance (Soh *et al.,* 2000; Duek *et al*., 2004).

The COP1/SPA complex also interacts physically with photoreceptors such as PhyA, PhyB and cryptochromes (Fig. 3). In red light, PhyA or PhyB signaling pathways are disrupted when PhyA or PhyB receptor is ubiquinated by COP1 with the involvement of 26S proteasome (Seo *et al*., 2004; Jang *et al*., 2010). PhyA stabilization is altered by transcription factor LAF1. Two possible mechanisms can explain how LAF1 is involved in PhyA stabilization. First hypothesis says that LAF1 could bind to ring finger domain of COP1 and could block or inhibit COP1 activity at specific phases during *Arabidopsis* development. The second supposes direct interaction between LAF1 and PhyA. This coupling prevents COP1 to ubiquitinate PhyA (Jang *et al*., 2010). Similarly in blue light, COP1 is associated with the degradation of CRY2 receptor (Shalitin *et al*., 2002). Conversely, blue light-activated CRY1 and CRY2 negatively regulate the function of COP1/SPA complex. CRY1 or CRY2 is binding to SPA1 protein, which results in dissociation of SPA1 from COP1 that suppresses the COP1 activity in regulation of gene expression in response to blue light (Lian *et al*., 2011, Zuo *et al*., 2011).

In opposite, light caused the inhibition of COP1 activity through the translocation to the cytosol (Fig. 2). The mechanism is not yet fully understood. Two possible explanations were provided. Pokhilco *et al*. (2011) hypothesized that COP1 inactivation is realized by the dissociation of CDD components, DDB1 and COP10, from the complex COP-SPA-CDD-COP9 signalosome. Another possibility was proposed by Balcerowicz *et al*. (2011) – the repression of COP1 activity is pursued due to a fall of SPA2 level. However, COP1 movement to cytoplasm exacted more than 24 hours exposition to light (Von Arnim *et al.,* 1994). Instead, the accumulation of transcription factors, targets of COP1, is much faster process (Pokhilco *et al*., 2011). In light, not only depletion of COP1 but also the post-translation modifications are contributed to the stabilization of transcription factors. Phosphorylation of HFR1 (Park *et al*., 2008) and HY5 (Hardtke *et al*., 2000) prevent their degradation.

The COP1 protein comprises three conserved parts (Fig. 4). At the N-terminal end Zn^{2+}-binding RING-finger motif is localized followed by the coiled-coil domain. This part alone is able to maintain the basal function during plant development. In the C-terminal half of the protein, WD-40 domain is responsible for the repression of photomorphogenesis. Other parts are NES and NIS (nuclear export and nuclear import signal, respectively), which ensure the COP1 translocation between the nucleus and the cytoplasm depending on the light condition (Torii *et al.,* 1998).

Tetramerization of COP1/SPA complex is realized thought COP1 coiled-coil domain. Transcription factors HY5 and HFR1 as well as all photoreceptors interact with the WD40 domain of COP1, while LAF1 binds to the RING-finger motif (Jang *et al.,* 2005).

Significant progress in research of COP1 signaling has been made in the last few years. Arguably, the complex domain structure is a result of diverse COP1 function in a variety of biological processes. However, many aspects of COP1 involvement still remain to be elucidated.

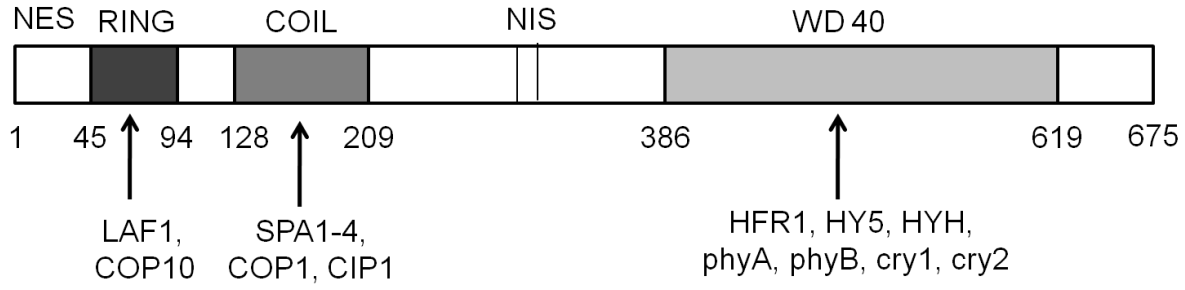


Fig. 4 *- COP1 structural domains and interacting proteins (according to Yi et al*., *2005)*

1.5 BORON

The key position of boron (B) for plant growth and development is known more than 85 years (Warington, 1923). In addition, B deficiency is the most widespread deficiency among all plant micronutrients (Loomis and Durst, 1992). The occurrence of insufficient boron was reported in 80 countries and 132 crop species (Shorrocks, 1997). Worldwide, the infinitesimal range between the deficiency and toxicity has a significant agronomic impact because boron affects mainly the high yield and quality of several crops (Brown *et al*., 2002).

The areas of high rainfalls as well as the soils derived from granite or sandstone rocks naturally contained the low levels of soil B. Under acid soil conditions, boron is more water-soluble and can be easily leached (Dear and Weir, 2004). The wide range of symptoms such as loss of fertility, reduced leaf expansion or cessation of root elongation is caused by the B deprivation. Their occurrence is dependent on the species and the age of the plant (Dell and Huang, 1997; Goldbach *et al*., 2001). Despite its essentiality for plants, boron at the higher concentration is toxic. Boron binds to the ribose moieties of ATP, NADH or NADPH and these

complexes then exhibit the metabolic disruption in the cell (Reid *et al*., 2004). Typical toxicity symptoms include chlorotic or even necrotic portions in the marginal region of mature leaves (Yau *et al*., 1995). The highest B concentration was found in soils derived from marine evaporites and marine argillaceous sediment. But also anthropogenic sources contribute the increasing B amount in soils. The water irrigation is one of the most fundamental contributors but wastes from surface mining, fly ash or industrial chemicals belong to the others. Whilst the lack of boron can be compensated by B-enriched fertilizers, ameliorating of high-B soils is more difficult. Boron in excess can be successfully removed from soil through the leaching action of water passing through and below the active plant root zone. Nevertheless, leaching is not a permanent solution due to the difficulties with the disposal of the leachates. The use of lime or gypsum is another possibility to manage toxicity problem (Nable *et al*., 1997). Recent studies try to generate the plant genotypes tolerant of high external B concentration (it will be discussed later).

But not many mechanisms of boron involvement in physiological processes were uncovered because it is extremely difficult to recognize primary from secondary or even tertiary function. The various postulated functions of B are based on the identification of a number of B deficiency symptoms. The most inspected boron role is determined in the primary cell wall. Boron is required for the stabilization of pectin polysaccharide rhamnogalacturonan II molecules through borate-diol bonding. This cross-link between two chains of monomeric RG-II molecules controls the porosity and strength of the cell wall and is necessary for standard plant growth (O'Neil *et al*., 2001, 2004). Boron is also involved in sugar transport, respiration and metabolism of IAA, carbohydrate and phenol, or in the stimulation of nucleic acid metabolism (Blevins and Lukaszewski, 1998). The discovery of B essentiality for the organisms lacking the cell wall indicates the broader role of boron, especially in the membrane transport network (Brown *et al*., 2002).

Boron occurs in the soil solution as boric acid or borate. The vascular plants receive boron through the roots in the form of uncharged boric acid (Marschner, 1995), but the B mobility vary among different plant species. Efficient re-translocation of B to the young tissues was observed in plants that transport sugar alcohols such as mannitol or sorbitol (Brown and Hu, 1996). Opposite to non-sugar alcohol-producing plant the boron distribution follows only the transpiration stream (Oertli, 1994). However, the accumulation of boron in the young and growing parts of the

leaves and stem out of the transpiration stream was reported even in these plants including e.g. *Arabidopsis thaliana*, *Triticum aestivum* or *Helianthus annuus* (Takano *et al*., 2001, Huang *et al*., 2001, Matoh and Ochiai, 2005). But this translocation was only attained under boron deficiency conditions which presumed that the plants are capable to regulate the boron transport within the plants and respond to changing boron condition in the soil solution (Tanaka and Fujiwara, 2008).

The high permeability of lipid bilayer for boric acid predicted for a long time that the passive diffusion mediated the only mechanism for the boron transport (Raven, 1980). After detailed characterization of B uptake in sunflower, Dannel *et al*. (2000) suggested that at lower B concentration the plants need the active B transport mechanism, whereas the passive diffusion is sufficient at higher B conditions. Thus, the evidence for the involvement of membrane proteins to boron transport emerged from the experiments investigated the sensitivity of *Arabidopsis thaliana* mutant *bor1-1* to B deficiency (Noguchi *et al*., 1997). Using positional cloning, Takano *et al.* (2002) then identified the boron transporter *BOR1* and also predicted six more related genes (*BOR2-BOR7*). In 2010 Fujiwara *et al.* confirmed that all of these genes code the proteins connected with boron transport activities. BOR1, the best known borate exporter, belongs to the bicarbonate transporter superfamily (SLC4) and is localized to the plasma membrane. Boron is transported via the BOR1 from pericycle cells to the xylem and under B limitation it is a critical process (Takano *et al*., 2002). Whereas B limitation caused the up-regulation of BOR1, under high boron condition the activity of BOR1 transporter is decreased because of degradation via endocytosis in the vacuole (Takano *et al*., 2005). Similar to *BOR1*, *BOR4* encodes an efflux-type of boron transporter. It is occurred mostly in a polar manner to the plasma membrane of the distal side of root epidermal cells. Overexpression of BOR4 improved significantly plant growth under boron excess conditions. Due to the effective B export from the roots, the boron level retained in the optimal concentration within the plant (Miwa *et al*., 2007). Transgenic rice plants expressing AtBOR4 showed the high tolerance to B toxic condition. The expression of BOR4 can be utilized to generation of high boron tolerant cultivars (Kajikawa *et al*., 2011).

Using microarray analysis Takano *et al.* (2006) identified NIP5;1, an aquaporin of the NIP (the nodulin 26-like intrinsic proteins) subfamily, which is up-regulated in boron-deficient *Arabidopsis* roots. By heterologous expression of NIP5;1 in *Xenopus* oocytes they demonstrated its role in boron transport. This boron transporter is

localized in epidermal, cortical and endodermal cells and ensure boric acid uptake to the cell. In 2008 Tanaka *et al.* also reported another protein NIP6;1 with the direct connection to boron transport. NIP6;1 might perform the function different from NIP5;1. NIPs are the members of MIP (major intrinsic proteins) family including aquaporins. NIP subfamily is specific for the plant. The nine *NIP* genes were observed in *Arabidopsis*. Some of them are multifunctional channels that are likely to transport electro neutral molecules such as water, urea or glycerol (Kaldenhoff and Fisher, 2006). NIPs are classified into two subgroups (I and II) on the basis of similarity or difference of aromatic/arginine (ar/R) region with that of protein Nodulin 26 (NOD26). Both NIP5;1 and NIP6;1 belong to the subgroup II. Their ar/R region has the larger pore diameter and is divergent from NOD26 in comparison with the NIP subgroup which possesses the conserved region of NOD26 (Wallace and Roberts; 2004, 2005).

Plants require the strict control of B concentration in the optimum range for the normal growth and development. The researches show that both B exporters and importers are important in this way in response to external boron conditions. B transporters are useful for improvement and generate tolerant plants to boron stress. Identification of boron transports also enabled the progress in the study of boron signalizing pathway. The aim of recent studies is to identify the genes of which expression is regulated by boron.

1.6 AUXIN

The first evidence about the existence of auxin, the important growth phytohormone, was described in 1881 by Charles and Francis Darwin in the impressive book The Power if Movement in Plants. The presence of auxin is indispensable for every aspect of plant growth and development, especially for the three essential processes in morphogenesis: cell elongation, proliferation and differentiation. Besides the proper embryogenesis, organogenesis, root initiation, vascular patterning, apical hook formation or apical dominance, auxin is involved in environmental responses of plant such as phototropism and gravitropism (McSteen, 2010; Tromas and Perrot-Rechenmann, 2010). Only the nanomolar concentration is required for the auxin function.

Two types of auxin are distinguished – natural and synthetic. The most abundant natural auxins are IAA (indole-3-acetic acid) and IBA (indole-3-butyric acid). Conversely, NAA (naphthylacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) are the most common used synthetic auxins. Due to auxin ability to stimulate growth, a number of auxin is artificially synthesized and utilized in agriculture (McSteen, 2010; Taiz and Zeiger, 2006).

Direct regulation of auxin amount within the plant is necessary for the proper plant growth and development as well as for the responses to environmental stimuli. Two distinct mechanisms, metabolism (biosynthesis, conjugation, catabolism) and transport within and between the cells are participated on this control.

The IAA biosynthesis occurs mainly in rapidly dividing and growing tissues. The inception of the synthesis is localized in the stem apical meristem and young leaves. However, other types of tissues are also capable to produce IAA (Ljung *et al*., 2001). The auxin IAA is structurally related to amino acid tryptophan. According to precursor, tryptophan or tryptophan precursor - indole-3-glycerol phosphate, two kinds of IAA biosynthesis pathways are realized – tryptophan-dependent or tryptophan- independent. Four different ways have been described from tryptofan to IAA. These ways include various intermediates but also often crossed. They are named by one of the intermediate - IAM (indole-3-pyruvic acid), IAOx (indole-3-acetaldoxime), TAM (tryptamine pathway), and IPA (indole-3-pyruvic acid) (review by Zhao *et al*., 2010, Mano and Nemoto, 2012). Only one tryptophan-independent IAA synthesis pathway was characterized – IAN (indole-3-acetonitrile).

Aforementioned, auxin is synthesized mainly in the apical meristem and therefore a mechanism for its transport to the target tissues must exist. Auxin is transported over long distance through the vascular system, or over shorter distance between the neighboring cells via the polar transport (PAT). All auxins are the weak acids that can exist in the form of ions with dissociated H^+ or as well as in the form of neutral molecules. Thus, their membrane permeability depends on the pH. In the apoplast (pH 5.5), 16% of the total amount of IAA is in the protonated form (IAAH), which is less polar and can pass the membrane spontaneously. Whilst the cytoplasm of the cells (pH 7) contains almost all of the IAA in the form of non-diffusible anion (IAA^-) that transport has to be regulated by the carriers: AUX1/LAX, PIN and ABC (ATP-binding cassette). These protein carriers are distributed asymmetrically on the plasma membrane (Zažímalová *et al*., 2010, Tromas and Perrot-Rechenmann,

2010). The group of AUX1/LAX transporters mediates auxin influx to the cell, namely IAA and 2,4-D. In opposite, NAA enters the cell passively by diffusion. AUX1 encodes the protein related to the amino acid permeases and together with three LIKE-AUX 1 (LAX1 – LAX3) proteins play an important role in plant growth, tropisms and organogenesis (Morris, 2000; Robert and Friml, 2009). Likewise AUX1/LAX, PIN proteins participate on direction of auxin flow within tissue. In *Arabidopsis*, eight PIN proteins are recognized that can be subdivided into two groups according to the length of a hydrophilic loop in the middle of their polypeptide chain. PIN1 - 4 and PIN7, members of the subgroup I, transport IAA, 2,4-D and NAA primarily from cytosol out of the cell. They show mostly polar plasma membrane localization. However, under different environmental stimuli, they can be re-localized to endosomal compartments via a transcytosis-like mechanism (Kleine-Vehn and Friml, 2008). Subgroup II with reduced hydrophilic loop represents PIN5, PIN6 and PIN8. They are localized in endomembranes, e.g. PIN5 is conversely contributed in auxin transport in the endoplasmic reticulum, thereby contribute to the maintaining of cytosolic concentrations of auxin and homeostasis (Mravec *et al*., 2009). ATP-binding cassette (ABC) is another large group of proteins, which apparently play major role in auxin transport. Members of ABCB subfamily transport auxin both outside and inside the cell against a concentration gradient (Zažímalová *et al*., 2010).

1.7 AUXIN AND BORON INTERACTION

According to dynamically fluctuating environment, the plants have been forced to modify their growth. This adaptation is an indispensable process to successfully complete their lifecycle. It comprises the coordination between internal control mediated by genes and external impact caused by environment cues (Walter et al., 2009). The connection between nutrition and hormone signaling pathways in the regulation of plant growth and development was clearly demonstrated (reviewed by Krouk *et al.,* 2011). Martín-Rejano *et al*. (2011) proved that low boron supply induced the increased activity of auxin transporter DR5-GUS in the primary roots of *Arabidopsis thaliana* and led to the considerable inhibition of root elongation. The proposed hypothetical model suggested that low levels of boron are related to increased auxin synthesis in the primary root meristematic region. Auxin is further transported via AUX1 to the elongation zone where it is accumulated which ensured

the inhibition of root cell elongation. These results are consistent with the research of others who documented the boron involvement in auxin transport and metabolism (Eaton, 1940; Dyarr and Web, 1961). Elevated boron added to the culture medium caused the decrease in the IAA level of the seedlings of *Triticum durum* in a dose-dependent manner (Gemici *et al*., 2002). The amount of IAA also dropped under boron stress condition in *Heliathus annuus* L. among the control plants grown in adequate boron supplement (Akcam-Oluk and Demiray, 2004). Likewise, in B-deficient plants of *Pisum sativum* L., IAA content was reduced and auxin export from the shoot apex was decreased compared to the plants grown under boron sufficient conditions (Wang *et al*., 2006). *Hordeum vulgare* cultivars showed the increased IAA levels when the boron was added (Ayvaz *et al*., 2012). Auxin supply is regulated by boron mainly to protect the IAA oxidase system through complexation of o-diphenol inhibitors of IAA oxidase, the main enzyme in auxin signaling pathway (Gupta, 2006). Hypothetical working model explaining how boron and light could influence hypocotyl responses to exogenous auxin was designed by Stočes *et al*. (2012). They proposed that the reduced auxin impact on *Arabidopsis* hypocotyl is associated with boron at high concentrations and blue light via the regulation of auxin transport within the cells (Stočes *et al*., 2012).

2 AIMS OF THE THESIS

1. To determine the mechanism of boron influence on plant photomorphogenesis and find out the role of E3 ubiquitin ligase COP1 in this process
   a) To study the expression of boron transporters BOR1 and NIP5;1 in mutant *cop1-5* with respect to different light conditions and boron concentrations
   b) To study the involvement of photomorphogenesis promoting transcription factors LAF1 and HFR1 in boron signaling pathway controlling the hypocotyl elongation
   c) To study the role of BL-photoreceptor ZTL1 in boron-regulated hypocotyl growth

2. To investigate how the negative regulator of photomorphogenesis COP1 via BOR1 influences the plant sensitivity to exogenous auxin and thus the hypocotyl elongation
   a) To study the boron effect on inhibition of hypocotyl elongation mediated by exogenous auxin 2,4-D or IAA in mutants *cop1-5*, *cop1-4* and *laf1*

3 MATERIAL AND METHODS

3.1 PLANT MATERIAL

Experiments were conducted on *Arabidopsis thaliana* (L.) Heynh. ecotypes Wassilewskija (Ws), Columbia (Col), Landsberg erecta (L*er*) and C24 and on the following mutant alleles: *cop1-5* (CS6259; T-DNA insertion; in background Ws), *cop1-5* (EMS; Col), c*op12-1* (CS3829; EMS; L*er*), *ztl1* (EMS; C24), *laf1* (T-DNA insertion; L*er*), *hfr1-201* (T-DNA insertion; Col) and *HFR1RNAi/laf1* (L*er*). Since mutants *cop1-5* and *cop12-1* are lethal in homozygous configuration, seeds of heterozygous plants were used for all experiments. The seeds of homozygous weak allele *cop1-4* were kindly provided by Xing-Wang Deng (Yale University, New Haven, USA), the seeds of *ztl1* were kindly provided by Steve Kay (The Scripps Research Institute, La Jolla, CA, USA) and the seeds of *laf1*, *hfr1-201* and double mutant *HFR1RNAi/laf1* were kindly provided by Nam-Hai Chua (Rockefeller University, NY, USA). All remaining mutants and ecotypes were ordered via TAIR (The *Arabidopsis* Information Resource, http://www.arabidopsis.org) and provided by NASC (The Nottingham *Arabidopsis* Stock Center, http://nasc.nott.ac.uk).

3.2 METHODS

3.2.1 MEDIUM PREPARATION

Basal solid MS medium (Murashige and Skoog, 1962) containing only microelements and macroelements was used for the experiments. The basal MS medium contained 0.1 mM boric acid (H_3BO_3), which corresponds to approximately 10 $mg.kg^{-1}$ boron typically found in many soils. MS stock solution without H_3BO_3 was also used as a control in the study of boron influence to auxin-induces changes in hypocotyl elongation. The MS medium was further supplemented with 1% (w/v) sucrose and 1mM MES 2-(*N*-morpholino)-ethanesulfonic acid). Before autoclaving the pH of the medium was adjusted to 6.1 with KOH and solidified with 0.7% (w/v) Phytoagar (Ducheva Biochemie; The Netherlands).

3.2.2 SEED STERILIZATION AND SOWING

The seeds of all genotypes used were surface-sterilized for twenty minutes in diluted commercial Savo original solution (1.5% sodium hypochlorite) supplemented with a drop of Tween 20. After that, seeds were rinsed eight-times with sterile distilled water and stratified at 4°C for 3 days in the darkness to promote and synchronize germination. Subsequently, the seeds were sown on Petri dishes (90 mm diameter) containing 20 ml of the sterile basal medium by an automatic pipette with modified tip. The tip was cut using a sterile scalpel to a width of aperture equal or slightly larger than the diameter of the *Arabidopsis* seed. Dishes with ca. 100 seeds (Fig. 5A) were sealed with microporous surgical tape (Batist s.r.o., CZ), wrapped in tinfoil and placed vertically into the temperature-controlled growth chamber (Microclima 1000, Snijders Scientific B.V., The Netherlands) at 23°C for 3 days.

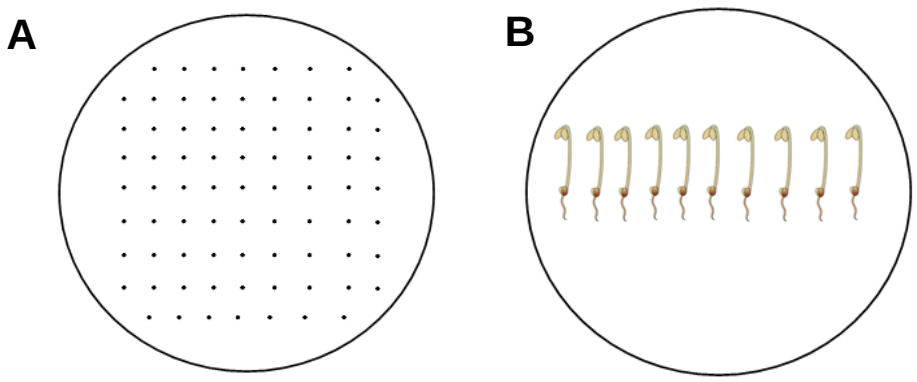


Fig. *5* – *The Petri dishes with the seeds (A) and transferred seedlings (B)*

3.2.3 SEEDLING GROWTH

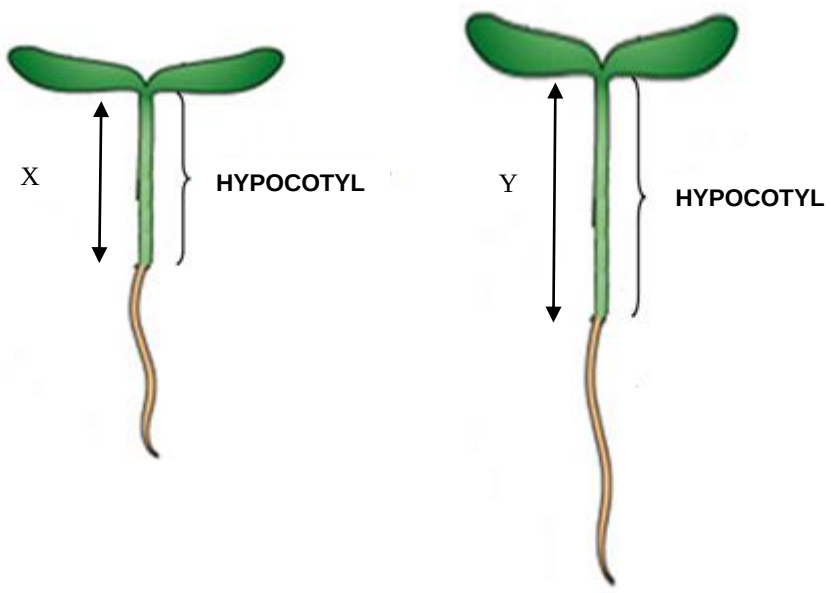
After the beginning of seed germination at least ten seedlings were transferred on a new MS medium using sterile needle. Additional boron (as boric acid) from a 0.5 M stock was added to produce final concentrations of 1, 2, 3, 5 and 10 mM B. For the study the effect of boron on auxin-induced changes in hypocotyl elongation, combination of boric acid at concentrations of 0, 0.1, 3.0 mM and auxin 2,4-D or IAA at concentrations of 0, 10^{-6}, 5.10^{-6}, 10^{-5} M were used (Fig. 5B). Petri dishes were sealed with the microporous surgical tape and placed vertically in the growth chamber with continuous red light (RL) or blue light (BL). For experiments in the dark,

Petri dishes were wrapped in a tinfoil and placed into the chambers. All seedlings were grown at 23°C for 10 days.

Blue light or red light was obtained using blue tubes Philips TLD-36W/18 or red tubes Philips TLD-36W/15, respectively. Maximum irradiance of BL was at 440 nm with total photon fluence rate 10 μmol m^{-2} s^{-1}. Maximum irradiance of RL was at 660 nm with total photon fluence rate 10 μmol m^{-2} s^{-1}. In both cases the intensity of radiation was 10 µmol.m^{-2}.s^{-1}. The light spectrum and intensity was measured by a portable spectroradiometer (model LI-1800, LI-COR, Lincoln, NE) calibrated by the Department of Biophysics at Palacky University in Olomouc.

3.2.4 MEASUREMENT OF SEEDLINGS GROWTH

The hypocotyl length of each seedling (Fig. 6) was measured using millimeter paper and tweezers. The obtained data were processed statistically and graphically in Microsoft Office Excel 2007. The change in seedling growth was calculated based on following formula (Fig. 6).



$$\textbf{growth change in } \% = \frac{X - Y}{X} \text{ x } 100$$

***Fig. 6** – The scheme and formula for calculation of changes in hypocotyl elongation (in %), where "X" states for hypocotyl length in the absence boron or auxin and "Y" states for hypocotyl length in the presence boron or auxin*

3.3 GENE EXPRESSION

For expression experiments, total RNA was isolated from hypocotyls of 10-day-old *Arabidopsis* seedlings grown in the temperature-controlled chamber under specific light and boron conditions. RNA extraction was performed using RNeasy Plant Mini Kit (Qiagen Inc., USA) according to the manufacture's instruction. The rest of genomic DNA was removed by digestion using RNase-free recombinant DNase I (Takara Bio inc., Japan) for one hour at 37°C. Phenol/Chloroform/Isoamyl alcohol extraction (25:24:1) was then performed to inactivate DNase I. After the sample spinning, the supernatant was transferred into new tube and 10 µl of 1M acetic acid, 275 µl of 96% EtOH and 0.5 µl of glycogene were added to the sample. The mixture was then stored overnight at -80°C. Afterwards, the precipitate was washed by EtOH, first by 70%, then 96%, spin each time for 5 minutes. Rest of EtOH was removed by pipette and pellet was dissolved in 20 µl of RNAse-free water after air-dried of EtOH for 5 min. The RNA purity was confirmed by PCR using genomic DNA *RSW1* oligonucleotides 5´-GGAGTCCTCGTGTTGAAGGA-3´ and 5´-AGGACCCAAAGGACCTGAT-3´ (Tab. 1). The PCR mixture contained 10.0 µl of RNAse free water, 4.0 µl of 5x GoTaq polymerase buffer, 2.0 µl of 1mM dNTP, 1.0 µl of 10 µM of each gene specific primer, 0.1 µl of goTaq polymerase (Promega, USA) and 2.0 µl of extracted RNA. RNA quantity and quality was measured by Nanodrop ND-1000 (Nanodrop technologies, USA).

Tab. 1 *- Conditions of testing RNA purity by PCR*

goTaq polymerase activation	94°C 5 min
Amplification 45 cycles	
Denaturation	94°C 30 s
Annealing	57°C 30 s
Extension	72°C 30 s
Final extension	72°C 5 min

Subsequently, c-DNA synthesis was performed using PrimeScript 1st strand cDNA Synthesis Kit (Takara Bio inc., Japan) according to the manufacture's instruction. Afterwards, RNAseH was added to each sample for 20 min at 37°C.

Finally, the cDNA product was directly used for PCR (Tab. 2). PCR reaction was performed with 11.0 µl of RNAse free water, 4.0 µl of 5x GoTaq polymerase buffer, 2.0 µl of 1mM dNTP, 1.0 µl of 10 µM of each gene specific primer (Tab. 3), 0.1 µl of goTaq polymerase (Promega, USA) and 1.0 µl of diluted cDNA. For each sample, three independent biological repeats were performed on thermocycler MJ Mini^{TM} (BIO-RAD, USA).

PCR products were size fractionated by electrophoresis in 1% (w/v) agarose gel stained with ethidium bromide. Received bands were evaluated by the software ImageJ to obtain relative gene expression.

Tab. 2 *– PCR conditions for the tested genes*

Gene	***EF1α***	***NIP5;1***	***BOR1***
goTaq polymerase activation	94°C 3 min	94°C 3 min	94°C 3 min
Amplification cycles	**30**	**35**	**33**
Denaturation	94°C 30 s	94°C 30 s	94°C 30 s
Annealing	57°C 30 s	64°C 30 s	55°C 30 s
Extension	72°C 30 s	72°C 60 s	72°C 60 s
Final extension	72°C 5 min	72°C 5 min	72°C 5 min

Tab. 3 *– Sequences of gene specific primers used in PCR*

Gene	**Primer sequence**
BOR1	5´- AATCTCGCAGCGGAAACG – 3´ 5´- TGGAGTCGAACTTGAACTTGTC– 3´
NIP5;1	5´- ACGGCTATACCTGCCAATTCTCCA – 3´ 5´- TAGCATTCGCTGCTCTAAGGCACT – 3´
EF1α	5´- AACAAGATGGATGCCACCAC – 3´ 5´- TGATCTGGTCAAGAGCCTCA– 3´

4 RESULTS

4.1 PHYSIOLOGICAL EXPERIMENTS

4.1.1 THE EFFECT OF BORON ON HYPOCOTYL ELONGATION

[redacted]

4.1.1.1 *cop1-5* HYPOCOTYLS

[redacted]

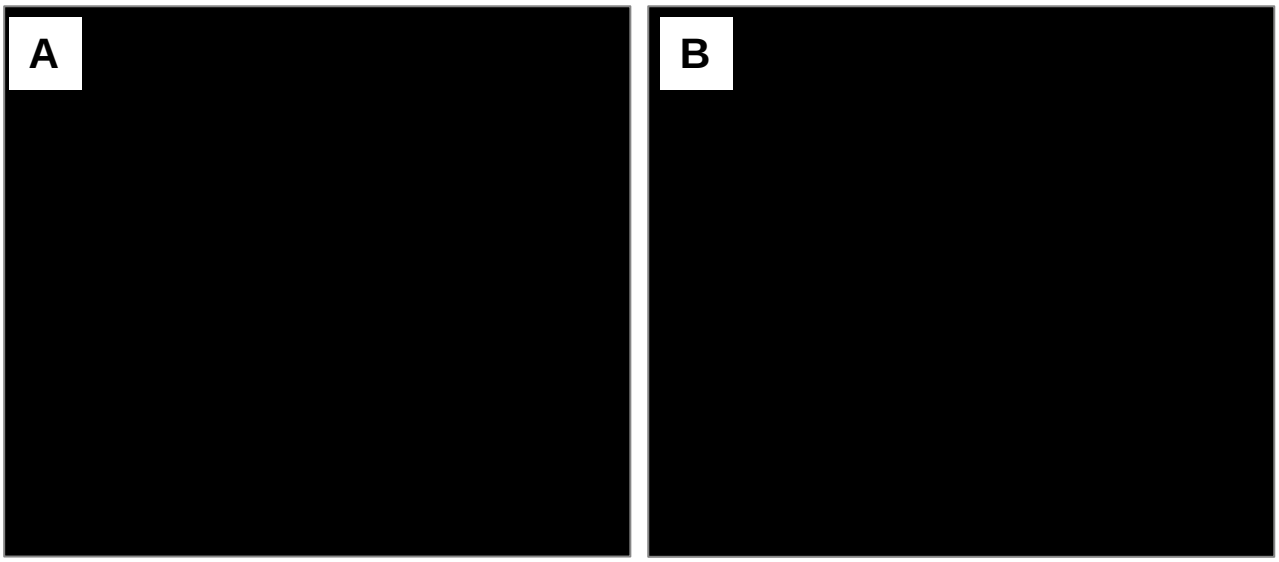


Fig. 7 *– The length of hypocotyls in WT (Ws) (A) - and mutant cop1-5 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 5 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

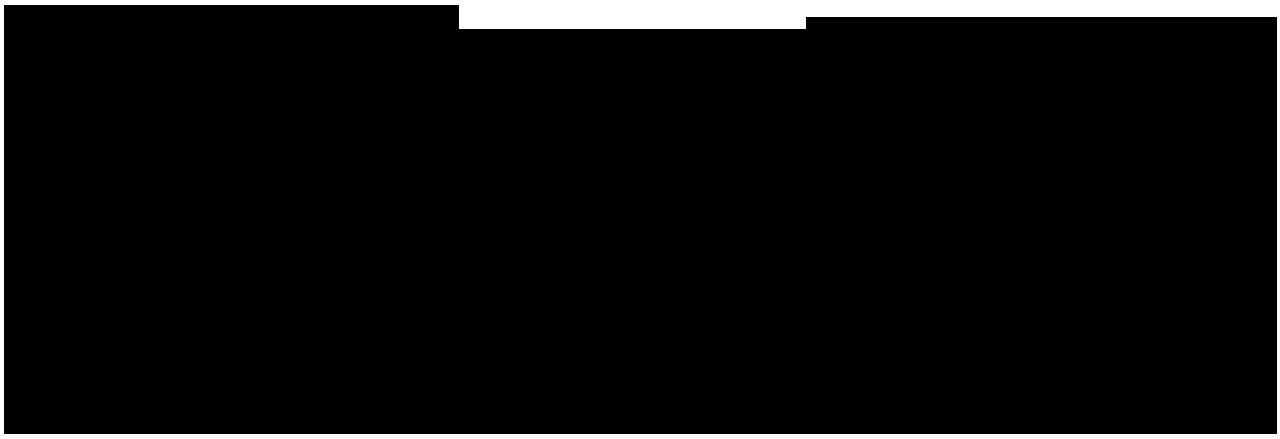


Fig. 8 *– The stimulation of hypocotyl elongation in WT (Ws) and the mutant cop1-5 at the boron concentrations of 2, 3 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 5 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.1.2 *cop12-1* HYPOCOTYLS

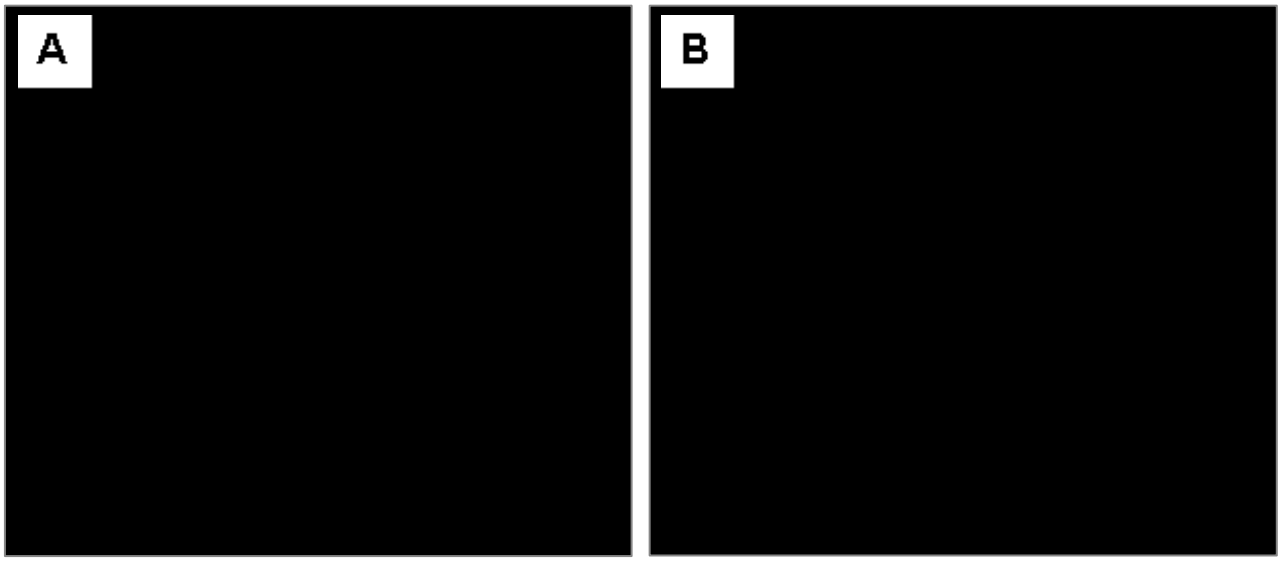


Fig. 9 *– The length of hypocotyls in WT (Ler) (A) - and mutant cop12-1 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

Fig. 10 *– The stimulation of hypocotyl elongation in WT (Ler) and the mutant cop12-1 at the boron concentrations of 2, 3 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.1.3 *ztl1* HYPOCOTYLS

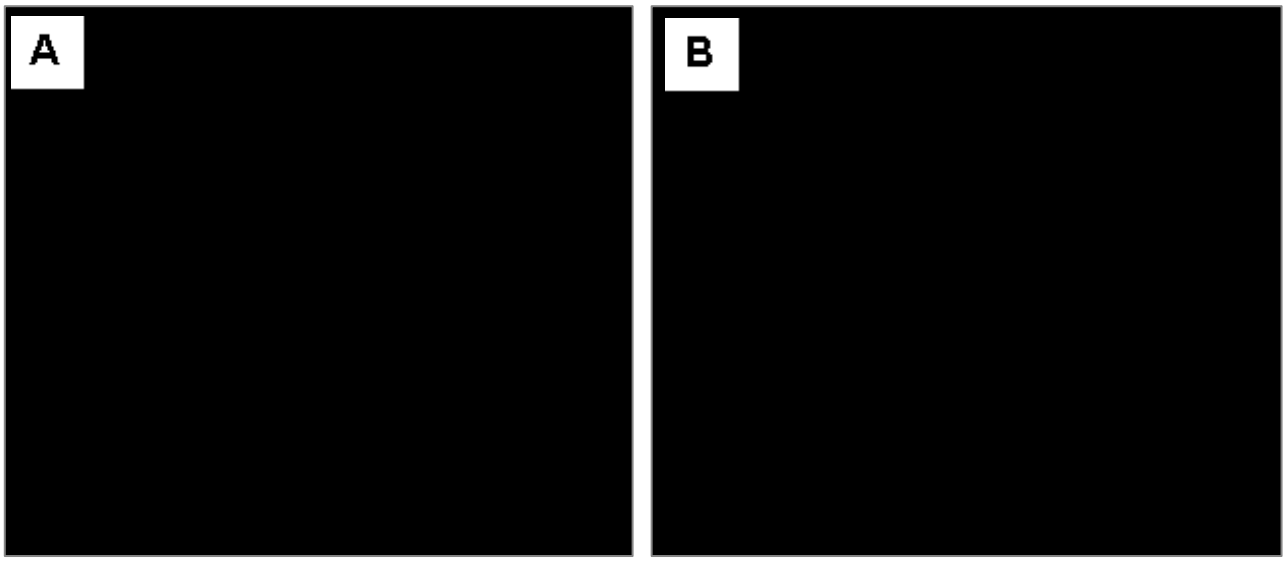


Fig. 11 *– The length of hypocotyls of WT (C24) (A), and mutant ztl1 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

Fig. 12 *– The stimulation of hypocotyl elongation in WT (C24) and the mutant ztl1 at the boron concentrations of 2, 3 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.1.4 *laf1* HYPOCOTYLS

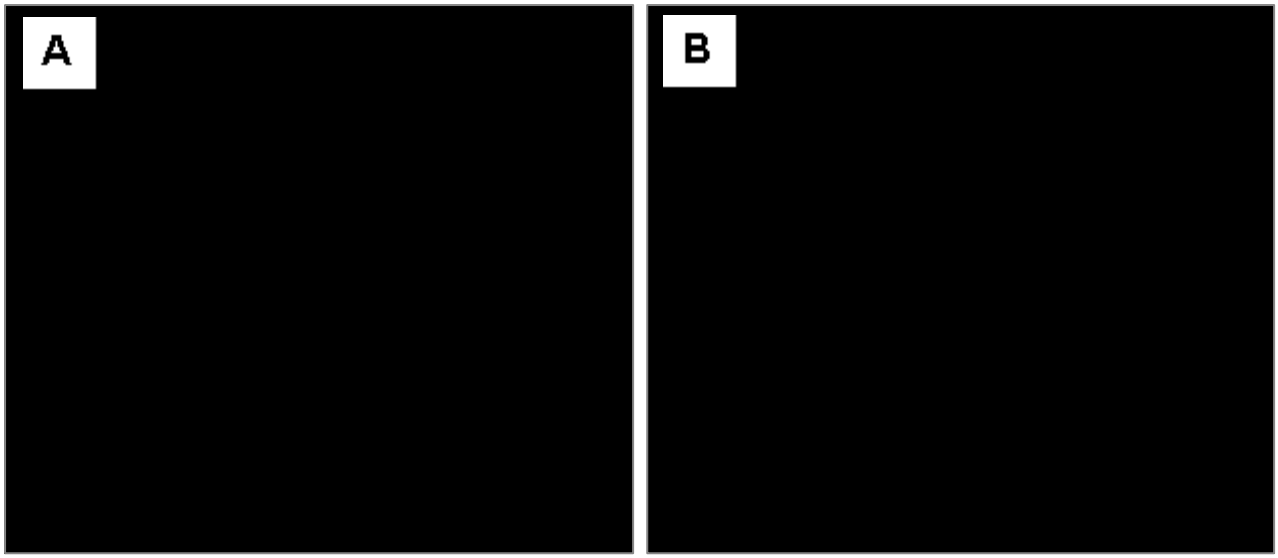


Fig. 13 *– The length of hypocotyls in WT (Ler) (A) - and mutant laf1 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

Fig. 14 *– The stimulation of hypocotyl elongation in WT (Ler) and the mutant laf1 at the boron concentrations of 2, 3 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.1.5 *hfr1-201* HYPOCOTYL

[redacted]

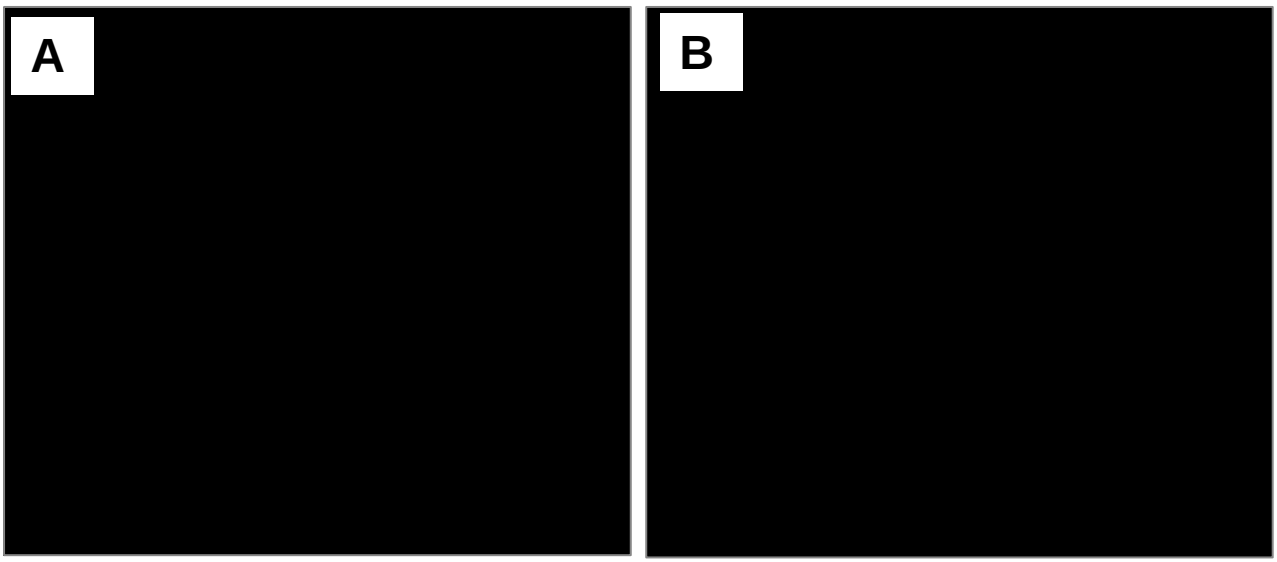


Fig. 15 *– The length of hypocotyls in WT (Col) (A) - and mutant hfr1-201 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

[redacted]

Fig. 16 *– The stimulation of hypocotyl elongation in WT (Col) and the mutant hfr1-201 at the boron concentrations of 2, 3 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.1.6 *HFR1RNAi/laf1* HYPOCOTYL

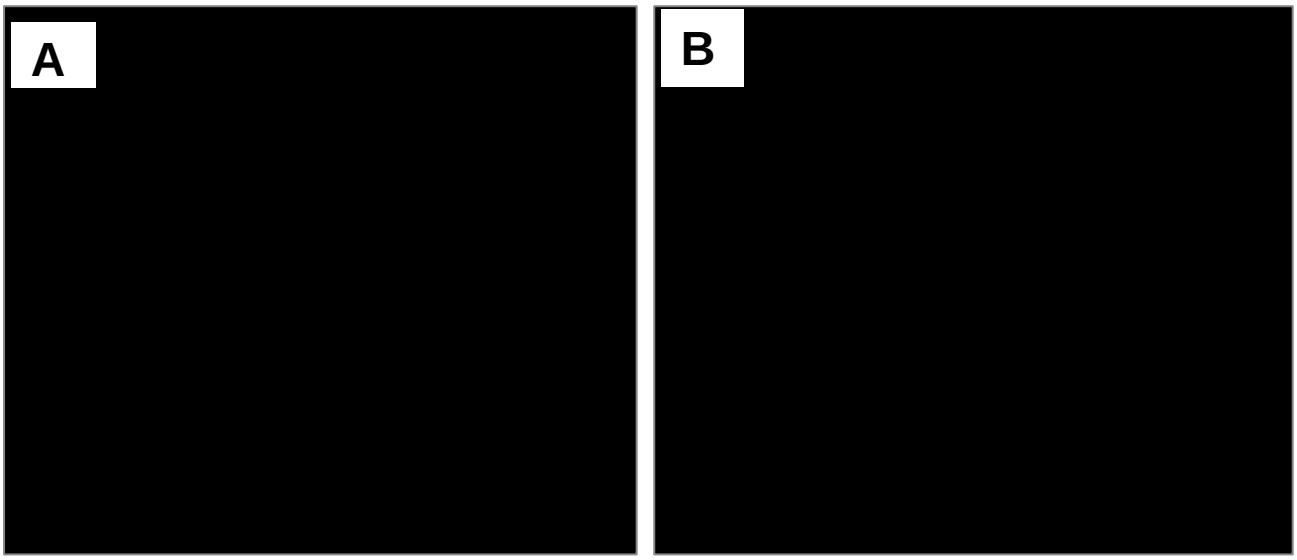


Fig. 17 *– The length of hypocotyls in WT (Ler) (A) - and double mutant HFR1RNAilaf1 (B) grown in the presence of boric acid at the concentration range from 0.1 to10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

Fig. 18 *– The stimulation of hypocotyl elongation in WT (Ler) and the mutant HFR1RNAilaf1 at the boron concentrations of 3, 5 and 10 mM in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

4.1.2 THE EFFECT OF BORON AND AUXIN ON HYPOCOTYL ELONGATION

[redacted]

4.1.2.1 *cop1-5* HYPOCOTYL

[redacted]

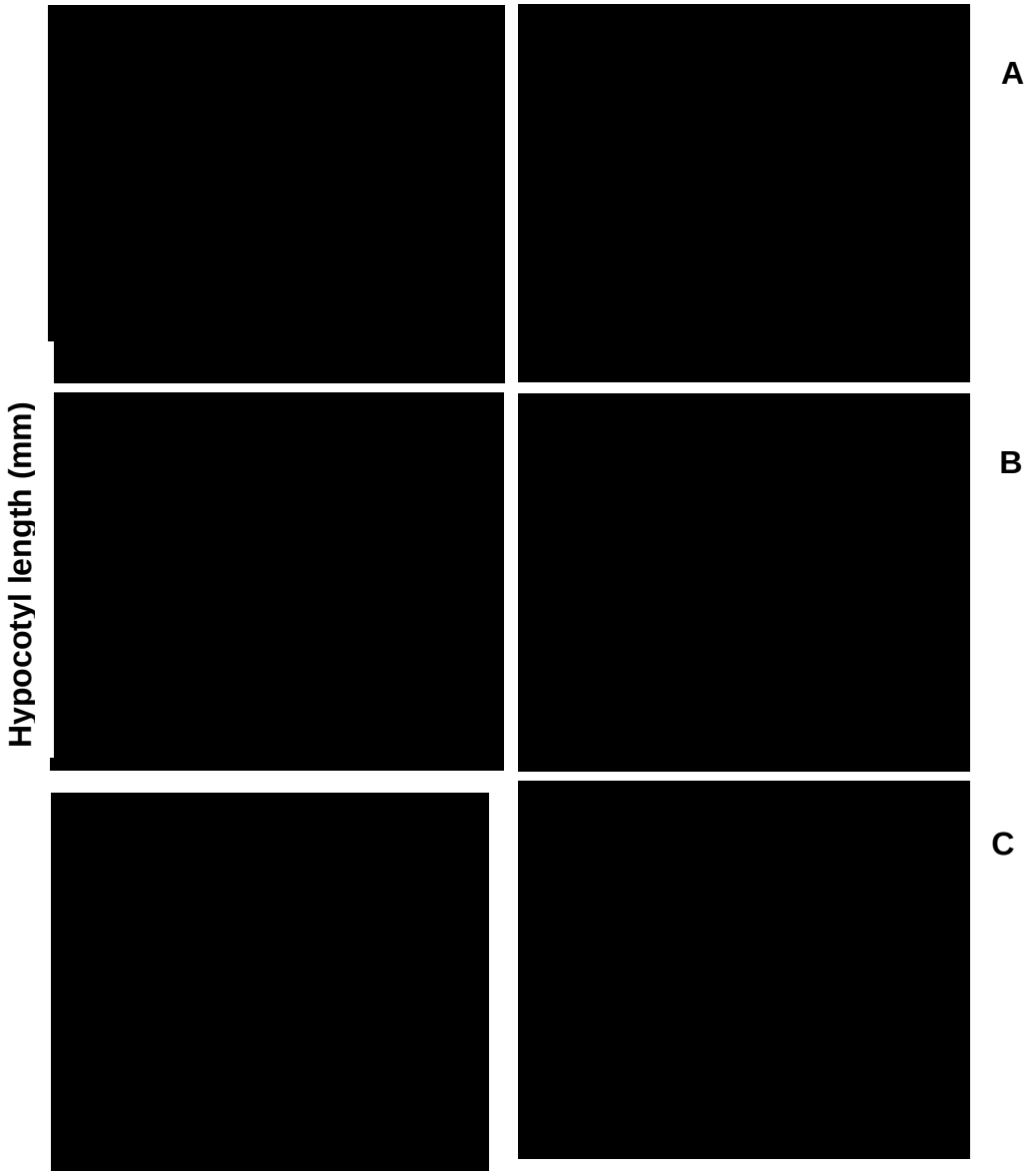


Fig. 19 *– The effect of auxin 2,4-D at the concentration range from 0 to 10^{-5} M on the hypocotyl length of WT (Ws) and mutant cop1-5 in the dark, blue or red light and in the presence of 0 mM (a), 0.1 mM (b) and 3 mM (c) boron. The data represent average ± standard errors obtained from 5 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

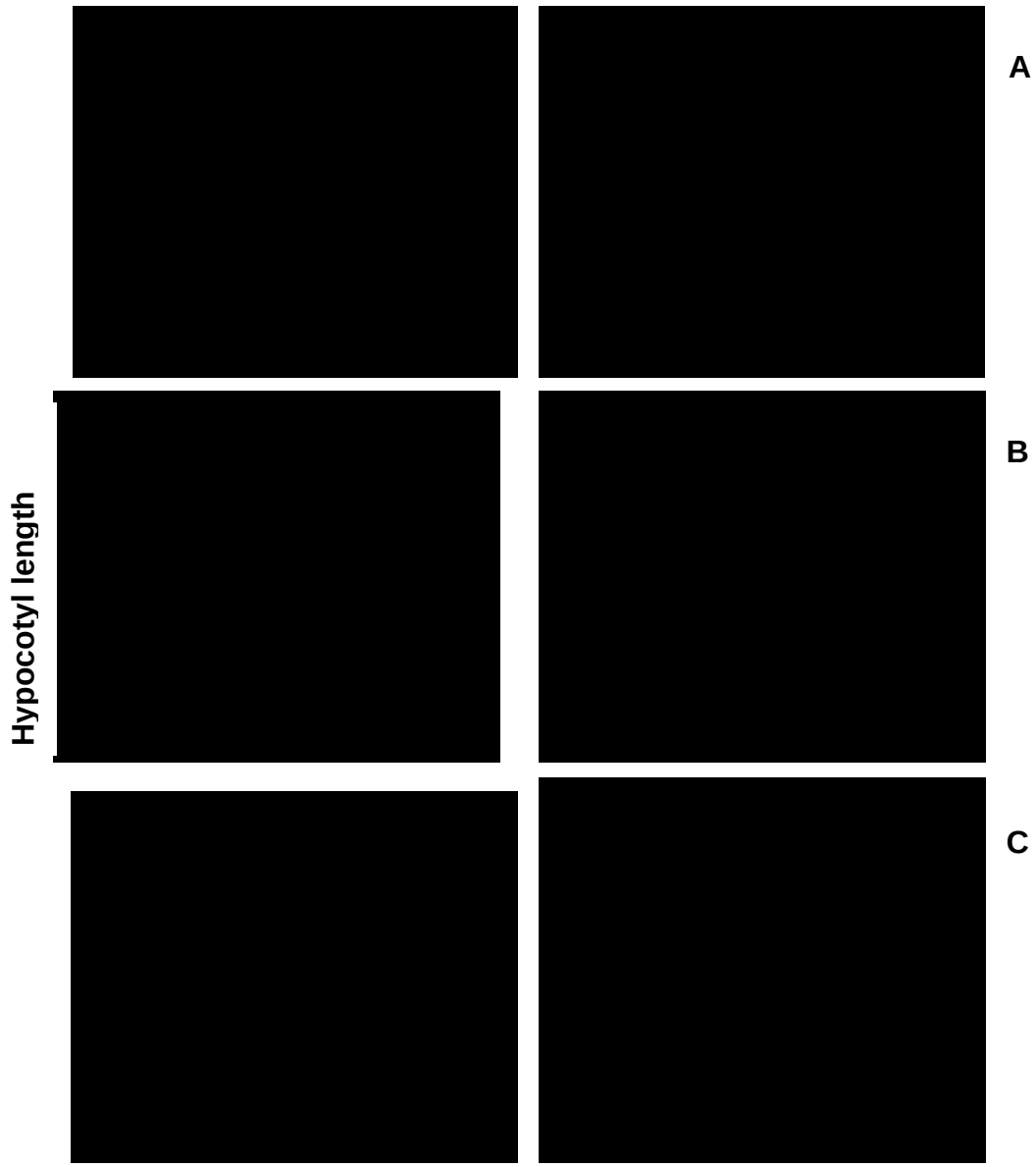


Fig. 20 *– The effect of auxin IAA at the concentration range from 0 to 10^{-5} M on the hypocotyl length of WT (Ws) and mutant cop1-5 in the dark, blue or red light and in the presence of 0 mM (a), 0.1 mM (b) and 3 mM (c) boron. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

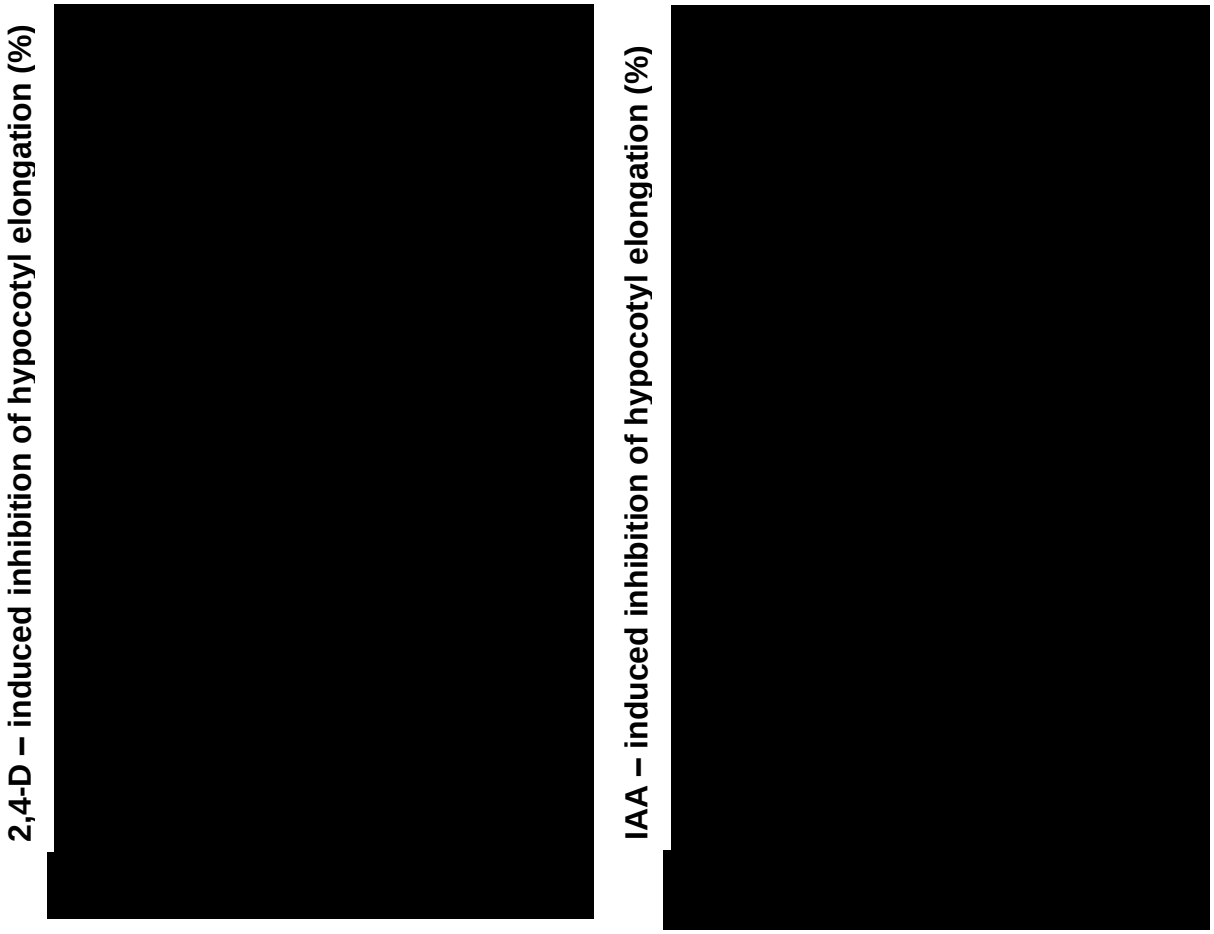


Fig. 21 *– The inhibition of hypocotyl elongation by 5.10^{-6} M auxin 2,4-D and IAA in WT (Ws) and the mutant cop1-5 at a concentration of 0, 0.1 and 3 mM boric acid in the dark, red and blue light. The data represent average ± standard errors obtained from 5 (2,4-D) or 3 (IAA) independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment*

4.1.2.2 *cop1-4* HYPOCOTYL

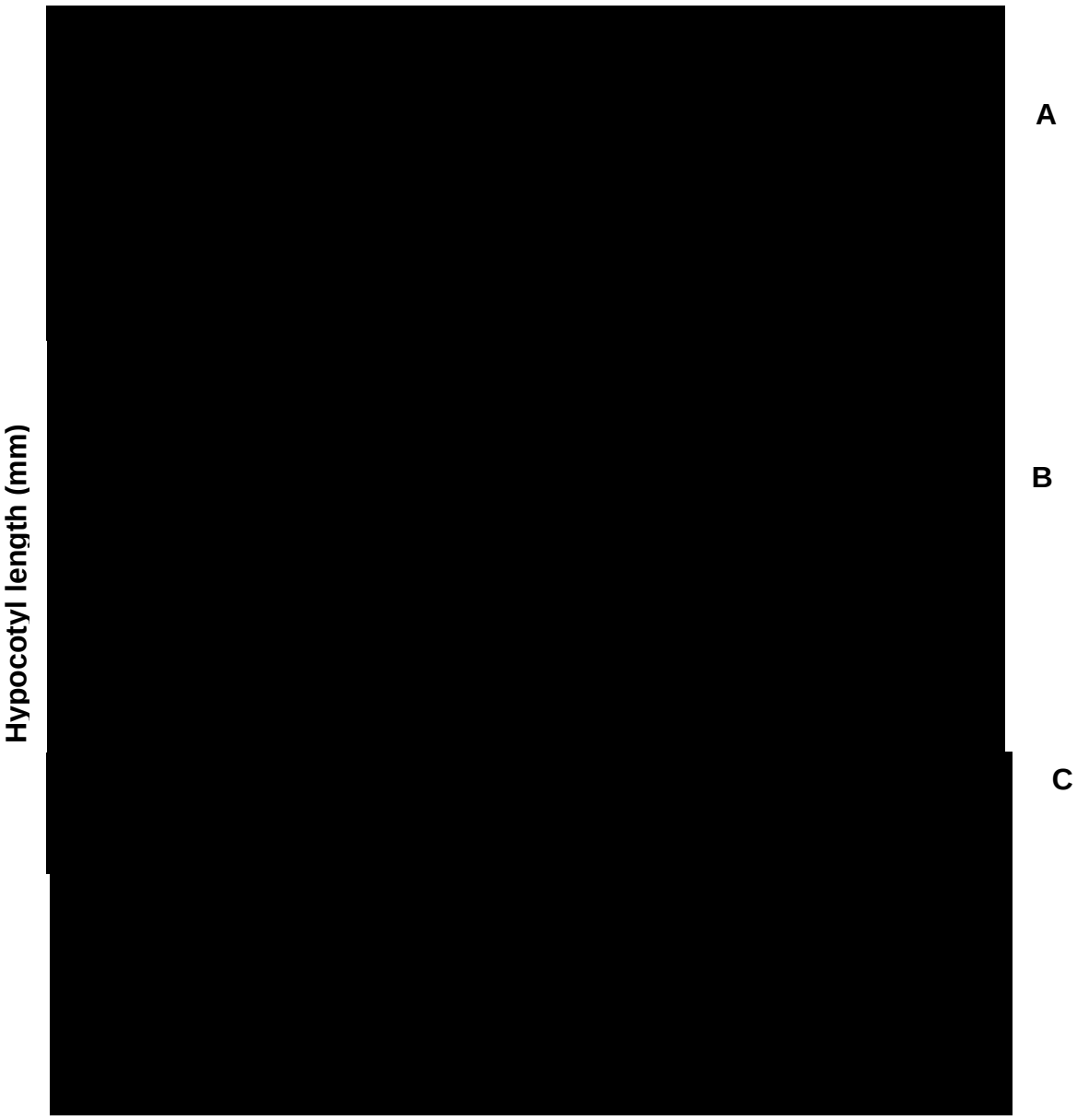


Fig. 22 *– The effect of auxin 2,4-D at the concentration range from 0 to 10^{-5} M on the hypocotyl length of WT (Col) and mutant cop1-4 in the dark, blue or red light and in the presence of 0 mM (a), 0.1 mM (b) and 3 mM (c) boron. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

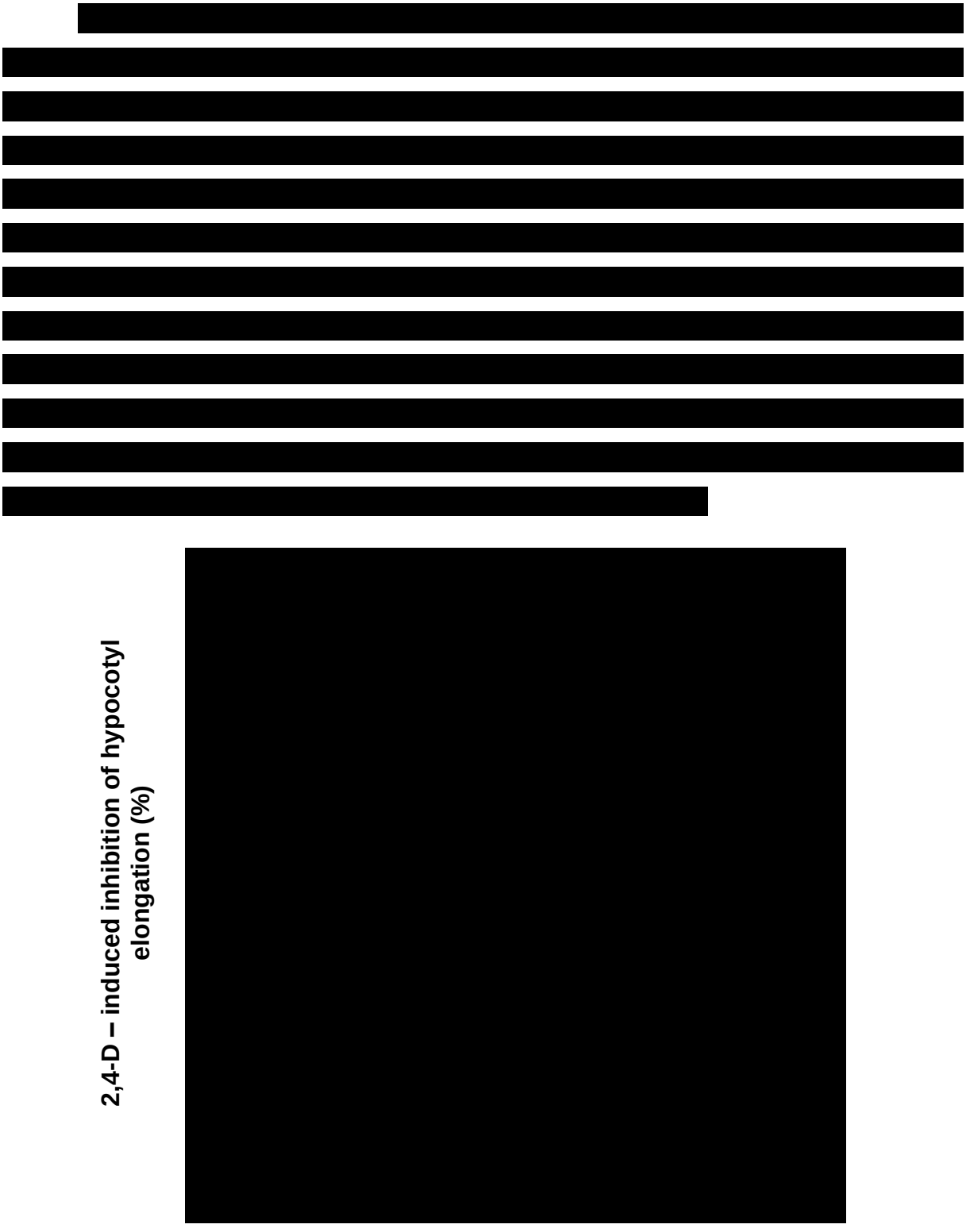


Fig. 23 *– The inhibition of hypocotyl elongation by 5.10^{-6} M auxin 2,4-D in WT (Col) and the mutant cop1-4 at a concentration of 0, 0.1 and 3 mM boric acid in the dark, red and blue light. The data represent average ± standard errors obtained from 4 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment.*

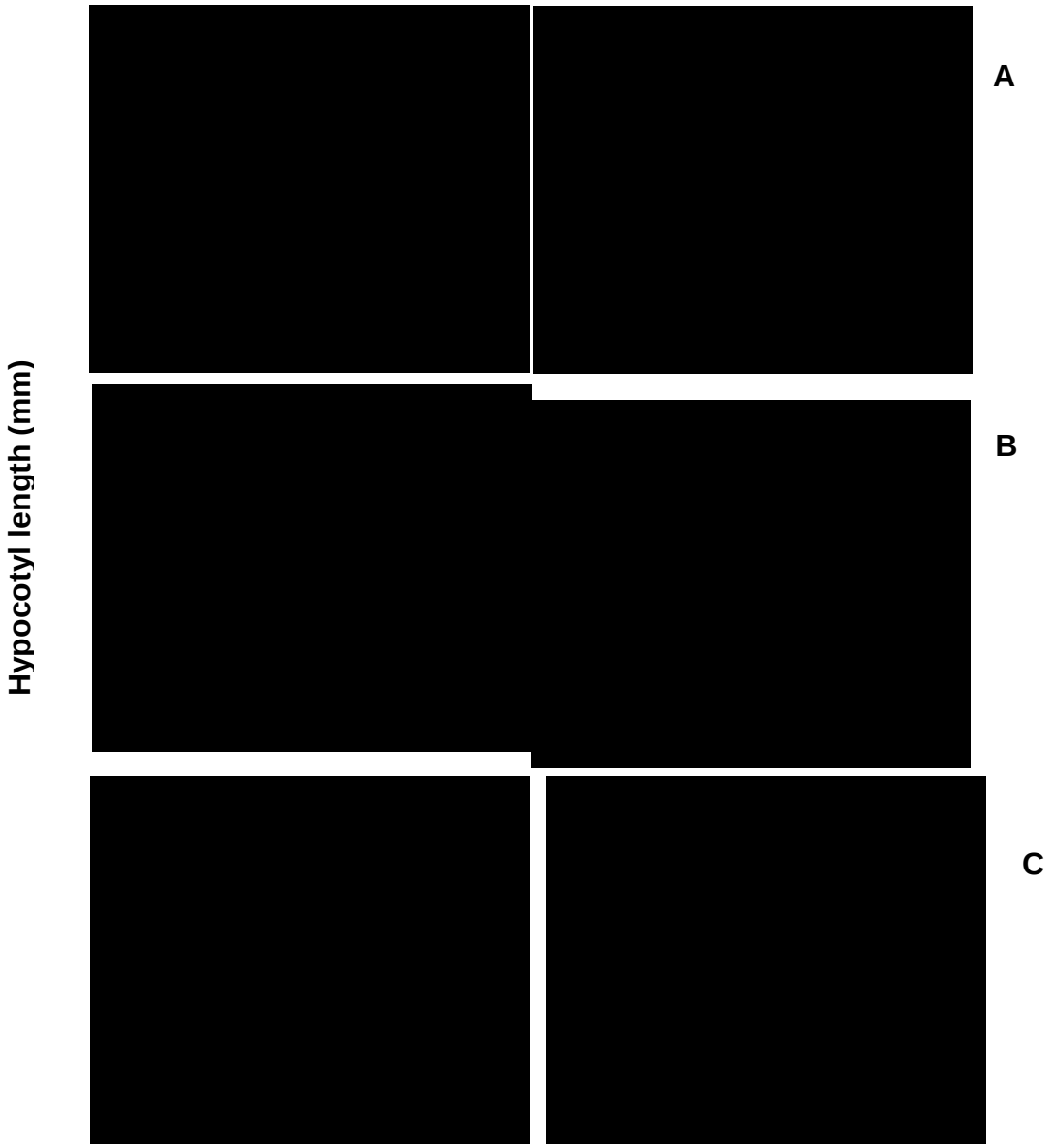


Fig. 24 *– The effect of auxin 2,4-D at the concentration range from 0 to 10^{-5} M on the hypocotyl length of WT (Ler) and mutant laf1 in the dark, blue or red light and in the presence of 0 mM (a), 0.1 mM (b) and 3 mM (c) boron. The data represent average ± standard errors obtained from 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment*

4.1.2.3 *laf1* HYPOCOTYL

Fig. 25 *– The inhibition of hypocotyl elongation by 5.10^{-6} M auxin 2,4-D in WT (Ler) and the mutant laf1 at a concentration of 0, 0.1 and 3 mM boric acid in the dark, red and blue light. The data represent average ± standard errors obtained 3 independent experiments, while at least 10 seedlings of each genotype were measured in each conditions and each experiment*

4.2 MOLECULAR EXPERIMENTS

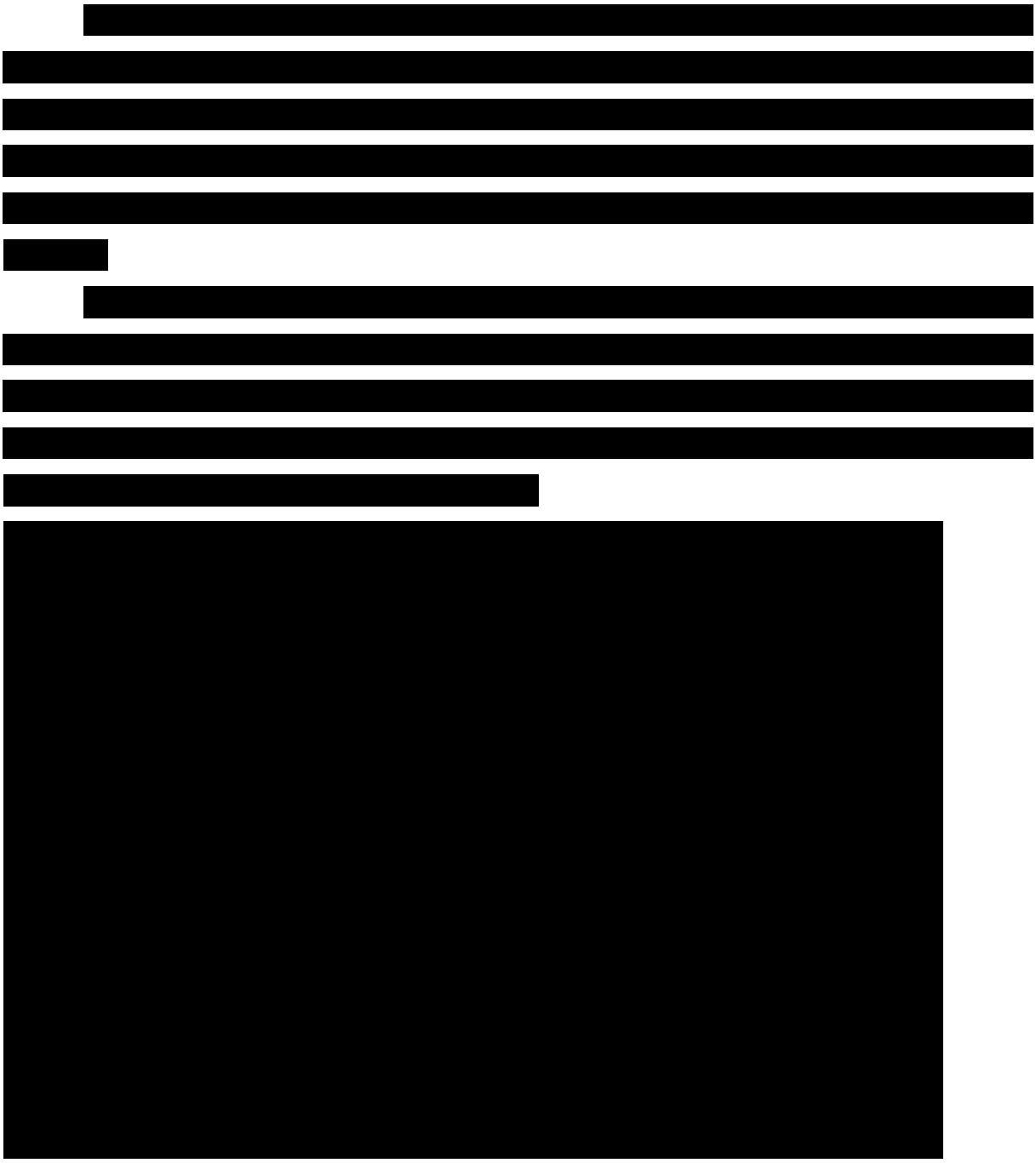


Fig. 26 *– Relative expression of NIP5;1 in hypocotyl of WT (Ws) and the mutant cop1-5 at the boron concentrations of 0.1, 3 and 10 mM in the dark, red and blue light. The data represent average of results ± standard errors obtained from 3 biological independent experiments. Results are replenished with image analysis of electropherograms of housekeeping gene EF1α.*

Fig. 27 *– Relative expression of BOR1 in hypocotyl of WT (Ws and the mutant cop1-5 at the boron concentrations of 0.1, 3 and 10 mM in the dark, red and blue light. The data represent average of results ± standard errors obtained from 3 biological independent experiments. Results are replenished with image analysis of electropherograms of housekeeping gene EF1α.*

5 DISCUSSION

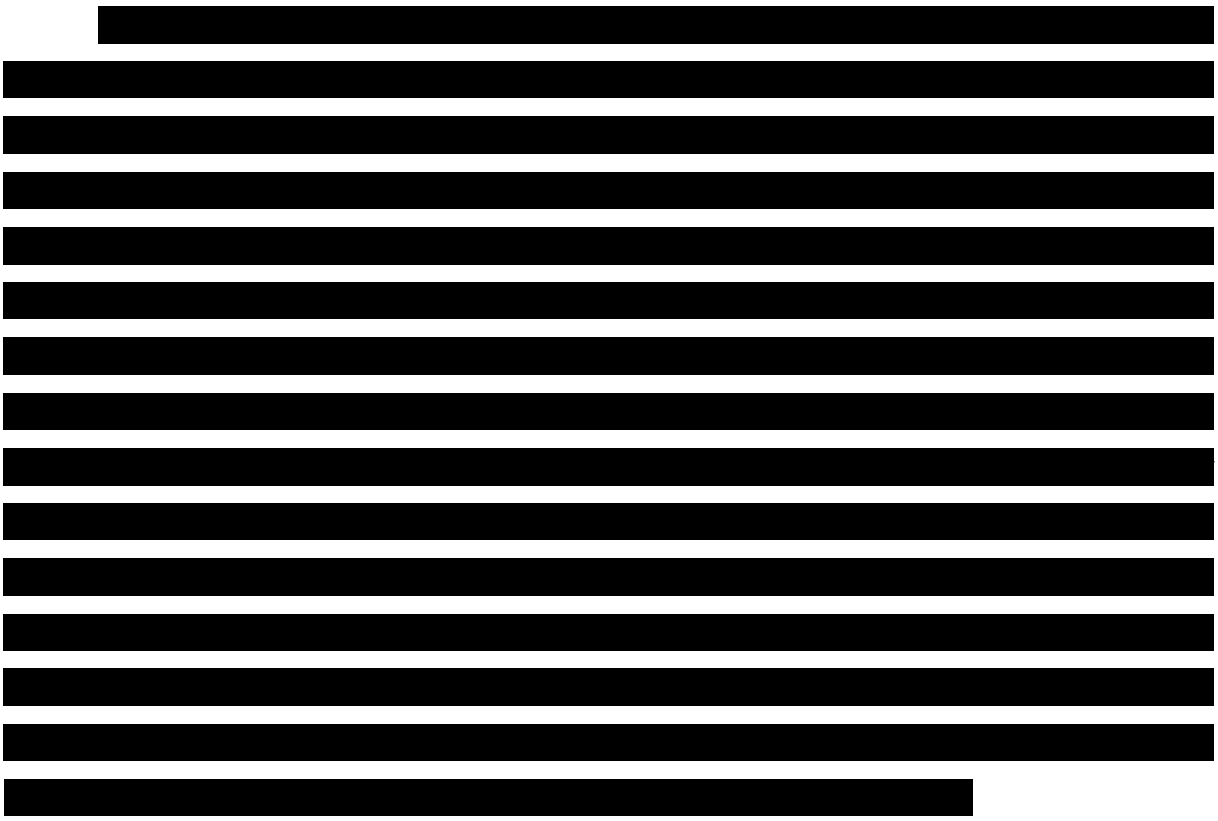
5.1 THE EFFECT OF BORON ON HYPOCOTYL GROWTH

[redacted]

Fig. 28 *– Scheme of COP1 involvement in boron-regulated hypocotyl elongation*

[redacted]

Fig. 29 *– Scheme of involvement of COP1 and photomorphogenesis-promoting factors LAF1 and HFR1 in boron-regulated hypocotyl elongation*



[redacted]

Fig. 30 *– Scheme of involvement of selected photoreceptors, COP1 and photomorphogenesis-promoting factors LAF1 and HFR1 in boron-regulated hypocotyl elongation*

5.2 THE EFFECT OF BORON AND AUXIN ON HYPOCOTYL ELONGATION

[redacted]

Fig. 31 *– Simplified scheme of the involvement of COP1 and LAF1 in boron-regulated response of hypocotyl elongation to exogenous auxin extended according to Stočes et al. (2012).*

5.3 ARABIDOPSIS ECOTYPES VARIABILITY

[redacted]

6 CONCLUSION

[redacted]

7 ABBREVIATIONS

2,4-D	2,4 – Dichlorophenoxy acetic acid
ABC	ATP - Binding Cassette
AUX1/LAX	AUXin1/like-AUXin1
ATP	Adenosine TriPhosphate
B	Boric acid
bHLH	basic Helix-Loop-Helix domain
BL	Blue Light
BOR 1 - 4	BORon transporter 1- 4
bZIP	basic leucine ZIPper domain
CAB	Chlorophyll A/B binding
cDNA	complementary DNA
CDD	complex of COP10, DET1 and DDB1
CHS	CHalcone Synthase
CIP1	COP1 Interactive Protein 1
COP 1 - 12	Constitutive Photomorphogenesis 1 - 12
CRY 1 - 3	CRYptochrome 1 - 3
CSN 1 - 9	COP9 Signalosome complex subunit 1 - 9
DDB1	Damage-specific DNA Binding protein 1
DET1	De-etiolated homolog 1
DNA	DeoxyriboNucleic Acid
DNAse	DeoxyriboNuclease
dNTP	DeoxyriboNucleotide
EF1α	Elongation Factor 1 α
EtOH	Ethanol
FKF	Flavin-binding Kelch repeat F box protein
HFR1	long Hypocotyl in Far-Red 1
HY5	elongated HYpocotyl 5
HYH	HY5 Homolog
IAA	Indole-3-Acetic Acid
IBA	Indole-3-Butyric Acid
LAF1	Long After Far-red light 1
LAX1 - 3	Like AUX 1 - 3
LKP2	LOV Kelch Protein 2
MS medium	Murashige and Skoog medium
NAA	NaphthylAcetic Acid
NADH	Nicotinamide Adenine Dinucleotide
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PCR	Polymerase Chain Reaction
NIP (5;1 6;1)	NOD26-like Intrinsic Protein
NOD26	NODulin 26
P_{fr}	Far red light absorbing form of phytochrome
PHOT 1 - 2	PHOTotropin 1 - 2
Phy A - E	Phytochrome A - E
PIN 1- 9	PIN - formed
P_r	Red light absorbing form of phytochrome
RG-II	RhamnoGalacturonan II

RNA	RiboNucleic Acid
RNAse	RiboNuclease
RL	Red Light
RSW1	Radially Swollen 1
SE	Standard Error
SPA 1-4	Suppressor of PhyA-105
UBQ	Ubiquitin
UV-B	Ultraviolet B
WT	Wild - Type
XTR7	Xyloglucan endoTRansglycosylase
ZTL	ZEITLUPE

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