BOTANIKER TAGUNG 2011

>DIVERSITY MAKES THE DIFFERENCE<

DBG

CERTIFICATE OF ATTENDANCE

This is to certify that

Yunus Effendi

has attended the

Botanikertagung 2011 Berlin

Diversity makes the difference - September 18-23, Berlin

Organizing Comitte

Prof. Dr. Reinhard Kunze

HUMBOLDT-UNIVERSITÄT ZU BERLIN

Freie Universität Berlin

DCPS Dahlem Centre of Plant Sciences

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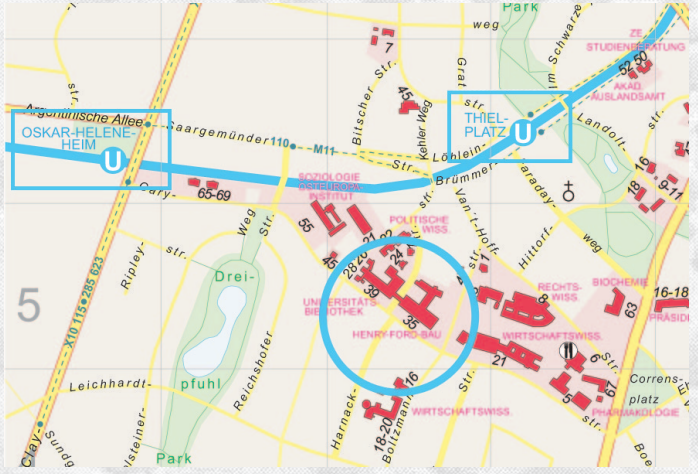
CONGRESS VENUE

The Botanikertagung 2011 will be held in the Henry Ford Building (HFB) on the campus of the Freie Universität Berlin in Berlin-Dahlem. The HFB offers state-of-the-art audio-visual facilities in all lecture halls and can be conveniently reached by subway from downtown Berlin or by bus/subway from Potsdam.

Henry-Ford-Bau (Reinhard Görner)

The conference dinner will be held in the Botanic Garden, Berlin-Dahlem. With its 126 acres, 16 greenhouses and more than 22,000 plant species, the Botanic Garden is internationally renowned for its extensive collections and unique exhibitions.



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The complete program, updates about the conference, a list of hotels and further information are available at the conference website

www.botanikertagung2011.de

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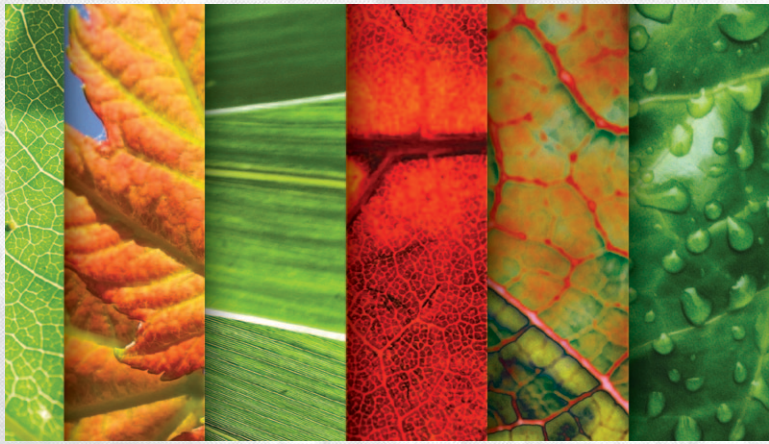


BOTANIKER TAGUNG **2011**

>DIVERSITY MAKES THE DIFFERENCE<

18 - 23 September 2011 - Berlin - Germany

www.botanikertagung2011.de

BOTANIKERTAGUNG 2011

The Botanikertagung is an international Botanical Congress held every two years under the auspices of the Deutsche Botanische Gesellschaft (DBG). The Botanikertagung 2011 is jointly organized by the Freie Universität Berlin and the Humboldt-University Berlin.

SCOPE

Under the title Diversity Makes the Difference the congress offers a multifaceted program that will bring together scientists and students from all fields of plant sciences, including phycology and mycology. A broad spectrum of topics will be covered, ranging from ecology to genome evolution and focusing on innovative and future-oriented concepts and approaches.

PRELIMINARY PROGRAM

Plenary speakers

Ian Baldwin (DE)
Philip Benfey (US)
Jeffry Chen (US)
Joanne Chory (US)
Erwin Grill (DE)
Eva Kondorosi (FR)
Peter Langridge (AU)
Robert Last (US)
Jian-Ren Shen (JP)
Kazuo Shinozaki (JP)
Klaas Jan van Wijk (US)
Dani Zamir (IL)
Daniel Zilberman (US)

Evening Public Lecture

Wilhelm Gruissem (CH)

Symposia

Please visit the conference website for further information regarding the confirmed keynote speakers.

Workshop: Seed Biology

SYMPOSIA TOPICS

- Developmental biology: Organ formation, patterning and differentiation
- Sexual reproduction and flower development
- Growth regulation: Cell cycle, expansion, division
- Organelles: Biogenesis, function, communication
- Plant respiratory control: hypotheses and mechanisms
- Plant mineral nutrition metabolism
- Secondary metabolism
- Membranes: Structure, functions and dynamics
- Plant cellular biology - from protein homeostasis to protein trafficking and targeting
- Photosynthesis: Electron transport chain
- Photo protection, photo inhibition and redox control
- Photosynthesis: Carbon assimilation, C4 and CAM plants
- Cyanobacterial photosynthesis
- Light sensing, light signaling, and circadian rhythm
- Rapid intracellular responses in (receptor-mediated) stress signalling
- Plant hormones: Metabolism, signalling and function
- Abiotic stress: Responses, acclimation, tolerance
- Biotic Stress: Host-pathogen interactions, attack and defence
- Global changes in terrestrial and aquatic ecosystems
- Molecular aspect of algae: From environment to physiology and cell biology
- Biotic stress: Plant defence against herbivores
- Plant-microbe symbiosis and mykorrhiza
- Crop improvement: Agronomic traits and optimized plant usage
- Green biotechnology
- Gene regulatory networks - transcriptional control
- Posttranscriptional control of gene expression: Non-coding RNAs, RNA processing and translation
- Epigenetics
- Heterosis
- New -omics technologies and applications
- Systems biology
- Molecular systematics
- Biodiversity and evolution: Genome stability, plasticity and remodeling
- Environmental context of evolution and speciation
- Evolution of secondary metabolism

SCHEDULE AND DEADLINES

24 January 2011	Online registration and abstract submission opens
31 May 2011	Deadlines for early bird registration and abstracts to be considered for oral presentations
15 July 2011	Deadline for abstract submission
9 September	Deadline for online registration
18 -22 September	Congress
23 September	Excursions

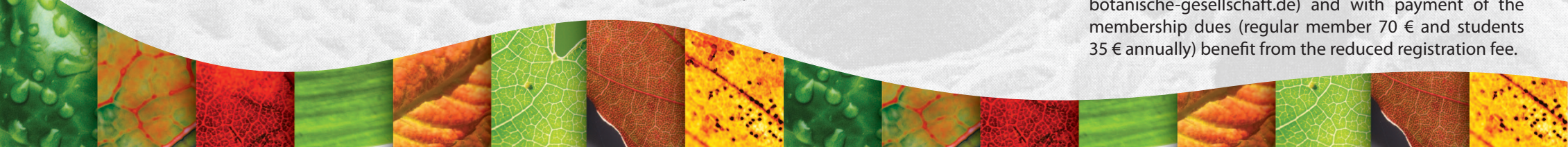
REGISTRATION

Online registration opens on 24 January 2011 at www.botanikertagung2011.de .

	Non-DBG-Members		DBG-Members		
	before 31 May	after 31 May	before 31 May	after 31 May	**day ticket**
Business	380 €	460 €	310 €	390 €	120 €
University/ Research	310 €	370 €	240 €	300 €	90 €
Student	135 €	175 €	100 €	140 €	45 €
Conference Dinner	35 €	35 €	35 €	35 €	

Payment is possible by bank transfer (preferred) or credit card. Students will be asked to show a valid student ID at the registration desk.

Non-members can become new members by applying to the Deutsche Botanische Gesellschaft (www.deutsche-botanische-gesellschaft.de) and with payment of the membership dues (regular member 70 € and students 35 € annually) benefit from the reduced registration fee.



GENERAL INFORMATION

LOCAL ORGANIZING TEAM

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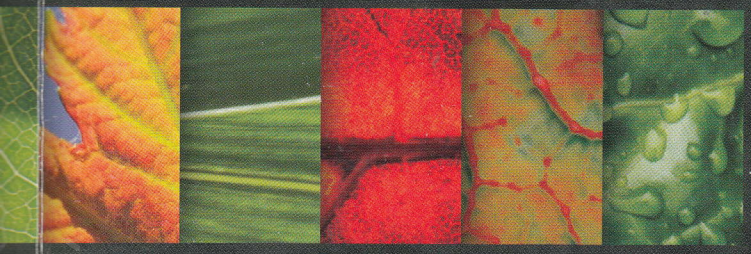

BOTANIKER TAGUNG 2011

CONFERENCE BOOK

POSTER ABSTRACTS | SESSION 27

S27 - POSTER -1

Patatin-related phospholipase A knockout mutants have defects in regulation of early auxin-induced genes

Corinna Labusch (1), Maria Shishova (2), Yunus Effendi (1), Günther F.E. Scherer (1)

(1) Leibniz University of Hannover; (2) University of St. Petersburg

In *Arabidopsis*, a family of ten phospholipase A genes has been identified and are involved in auxin and pathogen signaling (Rietz et al., 2010, Mol. Plant). Plant PLA activity is rapidly induced by different external signals and the PLA reaction products function as second messengers in plant signal transduction (Scherer et al., 2010, TIPS). Here we used the knockout mutants of all ten pPLAs to test the regulation of early auxin genes. Test genes were IAA-genes, SAUR-genes, genes involved in lateral root formation (Péret et al., 2009) and PIN-genes. Many of the lateral root genes and the SAUR genes showed a strong defect in gene expression in the pPLA knockouts after 10 µM auxin application (t=30min). In comparison, the transcription of pPLA genes themselves is not auxin regulated within 30 min. The pPLA knockouts did not show any phenotypes under normal growth conditions or when grown on auxin containing medium. In summary, the pPLA knockouts show a transient mis-regulation of early auxin regulated genes that mostly disappeared after 3 hours. Because the abp1/ABP1 mutant regulated none of early auxin-induced genes at 30 min we hypothesize that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi et al., 2011, Plant J.).

S27 - POSTER -2

The Cytokinin Receptors of *Arabidopsis thaliana* Localize Predominantly to the Endoplasmic Reticulum

Sergey N. Lomin (1), Klaas Wulfetange (2), Alexander Heyl (2), Georgy A. Romanov (1), Thomas Schmülling (2)

(1) Institute of Plant Physiology RAS; (2) Freie Universität Berlin

The plant hormone cytokinin is perceived by membrane-located sensor histidine kinases. *Arabidopsis thaliana* possesses three cytokinin receptors: AHK2, AHK3 and CRE1/AHK4. We investigated the subcellular location of the *Arabidopsis* cytokinin receptors by three different approaches. Cytokinin binding studies with plant membrane fractions separated by two-phase partitioning showed that in the wild type as well as in mutants retaining only single cytokinin receptors the major part of cytokinin-specific binding was associated with endomembranes. Cytokinin binding properties of plant membranes were similar to those found upon heterologous expression of receptors in a bacterial system. The transient expression of receptor-GFP fusion proteins or bimolecular fluorescence complementation analysis in leaf epidermal cells of *Nicotiana benthamiana* showed strong fluorescence of the endoplasmic reticulum (ER) network for each of the receptors. To detect Myc-tagged receptors in membrane fractions by immunoblotting, transgenic lines expressing recombinant genes under their own promoters were generated. Separation of the microsomal fraction by sucrose gradient centrifugation followed by immunoblotting showed a Mg^{2+}-dependent density shift of cytokinin receptors typical of ER membrane proteins.

S27 - POSTER -3

Transcript profiling of cytokinin action in *Arabidopsis* roots and shoots discovers organ-specific responses

Wolfram G. Brenner (1), Thomas Schmülling (1)

(1) FU Berlin, Dahlem Centre of Plant Sciences

Cytokinin regulates root and shoot growth in opposite ways: In shoots, it induces growth; in roots, it inhibits growth. Little is known about the assumed organ-specific regulation of gene expression involved in these differential activities. To get more insight into transcript regulation triggered by cytokinin in roots and shoots, we studied genome-wide gene expression in cytokinin-treated and cytokinin-deficient roots and shoots.We found by principal component analysis that the immediate-early response to cytokinin differs from the later response, and that the transcriptome of cytokinin-deficient plants is different from both the early and the late cytokinin induction response. A higher cytokinin status in the roots activated the expression of numerous shoot-specific genes, while a lower cytokinin status in the shoot repressed the expression of shoot-specific genes. This shift mostly affected nuclear genes encoding plastid proteins and indicated a cytokinin influence on the organ-specific transcriptome pattern independent of morphological organ identity.Novel cytokinin-regulated genes and new insights into the activities of cytokinin, including crosstalk with other hormones and translational control were found, which had escaped earlier discovery due to unspecific sampling.

S27 - POSTER -4

Characterization of CKX1-interacting HIPP proteins

Henriette Weber (1), Tomáš Werner (1)

(1) Freie Universität Berlin

The plant hormone cytokinin is an essential regulator of many physiological and developmental processes in plants. The concentration of cytokinin is effectively controlled through oxidative degradation catalyzed by cytokinin oxidase/dehydrogenase (CKX) enzymes. In order to understand the molecular mechanisms underlying the activity of CKX proteins, one of our approaches has been to seek for CKX-interacting proteins which could modulate the CKX enzyme activity, its subcellular localization or stability, or mediate a cellular activity primarily unrelated to CKX metabolic function. In a yeast two-hybrid screen, we found several HIPP proteins to interact with CKX1. The *Arabidopsis* HIPP protein family comprises 48 members and is defined by the occurrence of a heavy metal-associated domain (HMA) and an isoprenylation motif; a motif combination which is plant-specific. The function of HIPP proteins is currently unknown. Here, we present first results of our efforts to characterize the function of isolated HIPP proteins, including mapping of the interacting regions, determination of subcellular localization, and characterization of HIPP-overexpressing plants. The relevance of HIPP proteins for CKX activity and plant cytokinin responses will be discussed.

S27 - POSTER -9

Isolation and characterization of new point mutants of AUXIN BINDING PROTEIN1 (ABP1)

Yunus Effendi (1), Günther F.E. Scherer (2)

(1) Molekulare Ertragsphysiologie - Leibniz Universität Hannover; (2) Herrenhäuser Str. 2, D30419 Hannover, scherer@zier.uni-hannover.de

We showed that the heterozygous knockout mutant *abp1/ABP1* has defects in auxin physiology-related responses and lower transcript levels of early auxin-regulated genes (Effendi et al., 2011, Plant J. 65, 282).We designed two mutants, M7 and M8, by introducing a mutated cDNA, coupled to *35S* promoter, into heterozygous *abp1/ABP1* plants and screened for null wt gene transcription in the progeny. We also isolated transgenic plants expressing wt *ABP1* cDNA coupled to 35S (ABP1-OEX). M7 and M8 produced slightly shorter main roots but fewer lateral roots in response to auxin. They showed slowed hypocotyl phototropism and slowed root and hypocotyl gravitropism, which the ABP1-OEX did not show. M7 and M8 flowered early in SD but not ABP1-OEX. We also investigated the *abp1-5* (H94>Y94) point mutant. *abp1-5* shows slowed root gravitropism but hypocotyl phototropism or gravitropism was not changed and it flowered at the same time as Col in SD. qPCR of auxin-induced gene regulation in *abp1-5* shows it is a weak allele and first results on M7 and M8 show disturbed regulation of auxin-regulated genes. *ABP1* point mutants will be a valuable tool in auxin research.

S27 - POSTER -10

The role of cytokinin in regulating root system architecture in *Arabidopsis thaliana*

Ling Chang (1), Tanja Rublack (1), Eswar Ramireddy (1), Thomas Schmülling (1)

(1) Institute of Biology/Applied Genetics, Dahlem Centre of Plant Sciences, Freie Universität Berlin

Plant roots are responsible for nutrient and water uptake and provide physical support to the plant. Lateral roots (LR) make a considerable contribution to the root architecture and originate postembryonically, which is regulated by hormones and environmental signals. Cytokinin is a negative regulator of LR formation but the mechanisms of its action on LR development and its role in modulating root system architecture in response to environmental signals is still unclear.Phenotypic analysis of roots from cytokinin-deficient plants showed that most of them have increased LR density and altered distribution of LR primordia. In addition, analysis by VisuaLRTC revealed that expression of *CKX1*, *IPT3*, *IPT5*, *AHK2*, *AHK3* and *CRE1/AHK4* changed significantly during LR initiation and development. In order to get a first insight in the interplay with other factors regulating LR formation and growth, the root system of plants with an altered cytokinin status was analyzed on media containing various other hormones or soil-borne nutrients such as nitrogen and phosphate. The preliminary results showed that the cytokinin system is tightly interconnected with other signaling systems and that the cytokinin status predetermines the response to a number of different factors.

S27 - POSTER -11

Identification of cis-regulatory elements for gene regulation in response to cytokinin

Eswar Ramireddy (1), Andreas Pfeifer (2), Wolfram Brenner (1), Alexander Heyl (1), Thomas Schmülling (1)

(1) Dahlem Centre of Plant Sciences, Freie Universität Berlin; (2) Dahlem Centre of Plant Sciences, Freie Universität Berlin,

The identification of functional *cis*-acting DNA regulatory elements is a crucial step towards understanding the regulation of gene expression. In *Arabidopsis*, 11 B-type response regulators (B-type ARRs) regulate the transcription of their target genes in response to cytokinin. In fact, using the SRDX chimeric repressor technology, it was shown that B-type ARRs mediate most if not all of the transcriptional response to cytokinin. The B-type ARRs tested so far bind *in vitro* optimally to the core DNA sequence 5'-(A/G) GAT(T/C)-3'. However, so far the relevance of this *in vitro* binding sequence has not yet been demonstrated *in planta*. In the present study we attempted to identify specific target genes of one of the B-type ARR, ARR1, and the functionally relevant *cis*-acting element(s) for the cytokinin response. To this end, transcription profiles of wild-type, *arr1* mutant and *35S:ARR1* transgenic seedlings were compared and 24 genes were identified as putative specific target genes of ARR1. The promoter of one target gene (*ARR6*) was analysed in more detail by deletion analysis. The results not only confirm for the first time the functionality of above mentioned element *in planta* but also identified a novel *cis*-acting promoter region that functions co-operatively with the core element.

S27 - POSTER -12

Methyl-salicylate is a mobile form of salicylic acid in *Arabidopsis thaliana* infected by *Plasmodiophora brassicae*

Ivana Sola (1), Gordana Rusak (1), Jutta Ludwig-Müller (2)

(1) Faculty of Science; (2) Technische Universität Dresden

The mobile signals for systemic acquired resistance (SAR) in plants are plant-pathogen specific. It is known that salicylic acid (SA) enables the establishment of SAR in plants infected by biotrophic pathogens, but the nature of the long-distance mobile signal for SAR depends on the plant-pathogen system. The clubroot disease is one of the most devastating diseases affecting all the members within the plant family *Brassicaceae* by causing serious losses of vegetable crops worldwide. In this study, we wanted to investigate whether methyl-salicylate (MeSA) is a mobile form of SA implicated in this plant-pathogen interaction. We have chosen *Arabidopsis thaliana* as a host organism for *Plasmodiophora brassicae* – the causal agent of clubroot, because the infection process is fast and the resistance of *Arabidopsis* to *P. brassicae* is conferred by a small number of genes. Using a GC-MS method, we monitored the transport and the metabolism of exogenously applied deuterated salicylic acid and its derivative, deuterated methyl-salicylate, through the whole plant of healthy and *P. brassicae*-infected *Arabidopsis*. The results showed that MeSA is a mobile form of SA in *Arabidopsis* clubroots.

Patatin Related Phospholipase A Knockout Mutants have Defects on Regulation of Early Auxin-Induced Genes

Corinna Labusch[1], Maria Shishova[2], Yunus Effendi[1], Guenther F.E. Scherer[1*]

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Abstract

In Arabidopsis, a family of ten phospholipase A genes has been identified and are involved in auxin and pathogen signaling (Rietz et al, 2010, Mol. Plant). Plant PLA activity is rapidly induced by different external signals an the PLA reaction products function as secondary messengers in plant signal transduction (Scherer et al, 2010, TIPS). Here we used the knockout mutants of all ten pPLAs to test the regulation of early auxin genes. Test genes were IAA-genes, SAUR-genes, genes involved in lateral root formation (Perez et al, 2009) and PIN-genes. Many of the lateral root genes and the SAUR genes showed a strong defect in genes expression in the pPLA knockouts after 10uM auxin application (t=30 min), in comparison, the transcription of pPLA genes themselves is not auxin regulated within 30 min. The pPLA knockouts did not show any phenotypes under normal growth conditions or when grown on auxin containing medium. In summary, the pPLA knockouts show a transient mis-regulation of early auxin regulated genes that mostly dissapeared after 3 hours. Because the abp1/ABP1 mutant regulated of none early auxin-induced genes at 30 min we hypothezise that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi et al, 2011, Plant J.)

Keyword: Phospholipase, ATPLA-I, Gravitropism, Phototropism

Introduction

Phospholipase A (PLA) hydrolyses phospholipids either at the hydroxyl group of the C1 (PLA1) or of the C2 atom (PLA2) and liberates free fatty acids and lysophospholipids as products. Several structurally different enzymes can show this activity in plants (Scherer 2010; Scherer et al. 2010). These enzymes are the small (14 kDa) secreted PLA2 (sPLA2) (Ståhl et al. 1999), also found in fungi and animals, and the larger soluble patatin-related PLA (pPLA), which encompass the homologous soluble calcium-independent PLA2 (iPLA2) in animals (Six & Dennis 2000; Balsinde & Balboa 2005) and patatin-related PLA (pPLA) (Scherer et al. 2010). pPLAs hydrolyse fatty acids of both phosphoand galactolipids (Matos et al. 2001; Yang et al. 2007). The plant pPLA gene family is divided into three subfamilies (Holk et al. 2002; Scherer et al. 2010). The enzymes of the pPLA groups II and III with no additional domains have a molecular weight of around 50 ± 5 kDa. Proteins of subfamilies II and III do not have domains besides the enzymatic domain itself and can be distinguished by overall homology and their exon–intron structure. Group I in Arabidopsis

comprises only one gene (At-pPLA-I) having an additional N-terminal leucin-rich repeat (LRR) domain with a Gprotein-binding motif within this LRR domain, and a Cterminal domain with unknown function. Gene pPLA-I is the one gene most similar to the homologous so-called calciumindependent PLAs (iPLA) described for animals (Winstaed et al. 2000; Holk et al. 2002), and therefore, probably the evolutionarily oldest pPLA gene in plants.
The protein family of patatin-related phospholipases A, also referred to as PLP (La Camera et al., 2005), was named after the first sequenced homolog, patatin (Racusen, 1984; Andrews et al., 1988; Mignery et al., 1988). The AtPLA family can be classified further into three sub-groups according to amino acid sequence homologies and intron/exon structures (Holk et al.,2002). The location of each of the 10 AtPLA genes on the five Arabidopsis chromosomes is indicated by a Roman number followed by a capital letter when more than one pPLA gene exists on one chromosome (e.g. AtPLAIIA and AtPLAIIB). All of the pPLA proteins tested so far were shown to possess phospholipase A2 activity. The catalytic rates of AtPLAs were higher when galactolipids were used as substrates compared to phospholipids (Matos et al., 2001; La Camera et al., 2005; Yang et al.,2007). Although related to patatins, which are major storage proteins in potato tubers, AtPLAs lack an N-terminal signal peptide and are localized to the cytosol (Holk et al., 2002). Plant PLA activity is rapidly induced by different external signals and the PLA reaction products (released fatty acids and lysolipids) function as second messengers that regulate distinct proteins or downstream processes (Scherer, 1996; Meijer and Munnik, 2003). Thus, AtPLAs are thought to be important for early signal transduction events (Scherer, 2002; Ryu, 2004).

In contrast to the wealth of information on auxin-induced transcription and auxin transport (Paponov et al., 2008; Teale et al., 2008), little is known about signaling steps prior to transcription. Lysophospholipid levels increased after 1–2 min and fatty acid levels within 5 min of auxin application to parsley and soybean cell cultures (Scherer and Andre´, 1989, 1993; Paul et al., 1998). Such rapid profiles of auxin-induced PLA activity does not allow for biosynthesis of new proteins (Calderon-Villalobos et al.,2006), suggesting that PLA family proteins are post-translationally activated by auxin in planta. However, the identity of respective PLA enzymes and mechanisms by which the proteins are regulated has not yet been identified. Inhibitors of animal PLA2 enzymes also inhibited auxin-induced rapid release of FFA and LPL in cell culture cells, as well as elongation of zucchini hypocotyl segments, etiolation of Arabidopsis hypocotyls, and auxin-induced transcription (Scherer and Arnold, 1997; Paul et al., 1998; Holk et al., 2002; Scherer et al., 2007). The same compounds inhibited enzyme activities of two recombinant AtPLAs tested so far in vitro (Holk et al., 2002; Rietz et al.,2004). Therefore, sequence homology and biochemical characteristics of AtPLAs suggest them as likely candidates to signal auxin, and possibly other effector-mediated responses in the cytosol.
The T-DNA insertion mutants of pPLA-I investigated here, pplaI-1 (in Ws) and pplaI-3 (in Col), do not exhibit an obvious growth defect when grown in soil or on agar plates. However, we found the mutants to have a complex phenotype regulation of delayed early auxin-responsive genes, delayed gravitropism in darkness and decreased abrogation of gravitropism by far red (FR) in both alleles as well as and delayed phototropism in ppla-I-1. Coil formation was found in ppla-I-1 and phyB but not in ppla-I-3. Interestingly, both mutants showed a hypersensitive growth response in FR-enriched light pointing to compromised and low output of phyB signalling.

RESULTS

ppla-I mutants have a dynamic auxin response phenotype

When seedlings were grown in white (W) light or darkness, no obvious differences between the respective WT and pplaI-1 and ppla-I-3 mutant lines were observed. When tested on their response to auxin, no obvious differences in main root length or lateral root formation were found between mutants and respective WT. Therefore, the expression of early auxin activated genes in both mutant lines and WT was tested to monitor a potential auxin response (Fig. 1). Rapidity of expression of the candidate genes regulation by exogenous auxin was chosen as a main criterion (Effendi et al. 2011), and the selected candidate genes fall into three groups: IAA genes, some of which are involved in the formation of lateral roots (Fig. 1); SAUR genes (Fig. 3); and PIN2, PIN3, and GH3.5 (Fig. 2). Thirty minutes after the application of 10 M, 10 of 18 genes tested IAA in ppla-I-1 (Ws), and 11 in ppla-I-3 (Col) were induced significantly more weakly than in the corresponding WT.

In order to narrow down the time span during which pPLA-I could exert an influence on TIR1-directed transcription of early auxin genes, we investigated the expression of selected genes in the knockouts also after 10 min (Fig. 3). In both alleles, delayed up-regulation of IAA19, SAUR9 and SAUR15 could be observed even in this short time window. pPLA-I expression itself is not influenced by auxin after 10 min (Fig. 4), and only slightly after 30 min, excluding transcriptional regulation of pPLA-I as the cause of the diminished auxin-induction effect on early auxin-induced genes. When basal expression in untreated Col and Ws WT were compared, only PIN2 was clearly differently expressed (25×) while others were only about twofold differently expressed (IAA13, SAUR15, PIN3). Together, despite the absence of an obvious phenotype, both mutant lines show a clear phenotype in delayed auxin-induced expression of key auxin induceable genes.

Phototropism and gravitropism are impeded and root coiling is increased in ppla-I mutants

When both mutant lines were illuminated with unilateral blue light after 3 d in D (dark), only the pplaI-1 mutant showed a delayed phototropic response, but pplaI-3 did not, when compared with the respective WT (Fig. 5). After excluding sequence differences as potential cause for different phenotypes in the two alleles we speculated that the lack of phytochrome D (Aukerman et al. 1997) could be the cause for such dissimilarities. Phototropism can be promoted by switching off negative gravitropism in FR by phyA (Lariguet & Fankhauser 2004). Therefore we included phyA and phyB null mutants in the analysis. Indeed, the phyA mutant showed a reduced phototropic reaction, similar to ppla-I-1, whereas phyB reacted indistinguishable from WT (Fig. 5).

In the dark hypocotyls of pplaI-1 and ppla-I-3, the response to a gravitropic stimulus was considerably slower than the respective WT (Fig. 6a & b). After 24 h the average bending angle of the roots of dark-grown ppla-I-3 seedlings was statistically indistinguishable from WT, while the one of ppla-I-1 was slightly smaller than that observed of WT roots (Fig. 5c & d). Hypocotyl gravitropism is decreased by R and FR light (Robson & Smith 1996; Fankhauser & Casal 2004) and in pif1pif4 double and pif1pif3pif4pif5 quadruple mutants (Shin et al. 2009; Kim et al. 2011). The pif quadruple mutant hypocotyls are agravitropic in the dark indicating phytochromes as light receptors and their downstream transcription factors to be involved in gravitropism regulation. Therefore, we tested gravitropism in phyA and phyB seedlings under the same conditions as for ppla-I alleles. Only phyB hypocotyls showed a clearly reduced gravitropic response, whereas phyA roots and hypocotyls and phyB roots were indistinguishable from or very similar to WT in their responses (Fig. 6e–h). Furthermore, we investigated the inhibition of hypocotyl gravitropism by FR light (Fankhauser & Casal 2004). In phyB mutants, FR light abrogated gravitropism via phyA therefore hypocotyls grew random (Fig. 7). In fact, both ppla-I mutant lines showed a slightly

weaker abrogation of hypocotyl gravitropism than the respective WT, while the phyA mutant, because blind to FR, grew almost upright (Fig. 7b). In this test, both ppla-I knockouts displayed phenotypes resembling hard agar plates (Simmons et al. 1995) than Ws WT's tendency.

DISCUSSION

ppla-I mutants have a dynamic auxin phenotype similar to abp1 receptor mutants

Despite a lack of obvious morphological response to exogenous auxin in the knockout mutant lines compared with corresponding WT, we found clear defects in both knockout mutant lines in auxin-induced gene regulation using a set of selected genes as a physiological test (Fig. 4). This is reminiscent of our findings on the other investigated abp1 and eir1/pin2 mutants (Effendi & Scherer 2011; Effendi et al. 2011, 2013), and the other nine ppla null mutants that were tested the same way (Labusch et al. 2013). In abp1/ABP1 seedlings, all auxin-regulated genes were mis-regulated while in eir1/pin2, 9 of 12 were affected. In the case of both pplaI alleles described here, 11 of 18 auxin-regulated genes were affected. Auxin-induced inhibition of pPLA-I transcription was minor after 30 min (Fig. 4e), so that transcriptional regulation of pPLA-I itself as a component of this signalling appears rather unlikely (Fig. 4d). In contrast to the pPLA-I gene itself, whose expression was not changed within 10 min, we already found after 10 min, delayed transcription of early auxin-induced genes in ppla-I alleles (Fig. 4d). A transcriptional regulation by auxin of pPLA-I should be controlled by TIR1, but was not observed. Obviously, auxin-induced events elicited in less than 10 min need a receptor other than TIR1 (Scherer 2011;Scherer et al. 2012; Labusch et al. 2013). Therefore, our data rather indicate an auxin triggered post-transcriptional activation/inactivation event on pPLA-I to be evoked in less than 10 min.The strikingly similar phenotypes of abp1/ABP1 and abp1–5 (Effendi & Scherer 2011; Effendi et al. 2011, 2013) and the ppla-I alleles here, and the proven capacity of ABP1 to trigger rapid auxin responses (Scherer 2011; Scherer et al. 2012) favour ABP1 as the respective receptor although the specific mechanism remains to be uncovered. Moreover, the co-localization of pPLA-I (Fig. 3B, d–g; C, a,b,e,g) and ABP1 (Klode et al. 2011) in the plasma membrane, perinuclear ER and nuclear membrane is a precondition for their functional association.

Gravitropism

Gravitropic and phototropic bending involve differential growth regulation by auxin redistribution. Redistribution of auxin transport is mainly dependent on mobile PIN proteins, more specifically PIN2 and PIN3. In darkness, defects in gravitropism in pin2 and pin3 originate mostly as a consequence of disturbed gravisensing and subsequent misregulation of PIN2 and PIN3 subcellular localization and hence, disturbed lateral auxin transport (Friml et al. 2002;Abas et al. 2006; Ding et al. 2011). Mis-regulation of early auxin-induced genes during the gravitropic response could be another reason for defects in gravitropic responses. Up-regulation of PIN3 transcription by auxin was delayed in both mutant lines and may contribute to delayed gravitropism in our experiments. Other key auxin-dependent genes like ARF7, IAA19 and SHY2/IAA3 are supposed to be involved in hypocotyl gravitropism (Kim et al. 1998; Soh et al. 1999; Harper et al. 2000; Liscum & Reed 2002; Tatematsu et al. 2004). PIN3, IAA19, IAA3 and SAUR15 (Fig. 4a–d) were mis-regulated in ppla-I-1 and ppla-I-3. These components have been implicated to regulate growth asymmetry in response to lateral auxin transport carried out by PIN2 and PIN3 (Friml 2003).

Gravitropism is abrogated by FR and R (Poppe et al. 1996; Robson & Smith 1996; Correll & Kiss 2005), and abrogation light is mediated by phyA and phyB (Fankhauser & Casal 2004).A delayed gravitropic response of hypocotyls grown in darkness was found in both ppla-I lines and in phyB, but to a much lower extent in phyA, meaning that in this experiment, gravitropism, was not influenced by light and yet ppla-I alleles responded in a manner similar to a phyB mutant. We point out, however, that in a phyB signalling mutant possessing no PIF1 or PIF3 transcription factors, gravitropic orientation is also disturbed in the dark (Shin et al. 2009). The same phenotype is much stronger in the quadruple loss-offunction mutant pif1pif3pif4pif5 in both darkness and light (Kim et al. 2011). Further components downstream from phyB, which act in the regulation of hypocotyl orientation are GIL1 (Allen et al. 2006) and PKS4 (Schepens et al. 2008). Here both ppla-I alleles show a weak phenotype similar to the phyA mutant. This observation might indicate a role of pPLA-I in phyA signalling although low phyB signalling output cannot be excluded. As cytoplasmic phyA signalling has been described (Rösler et al. 2007, 2010) and an interaction of phyA and PHOT near the plasmamembrane has been observed (Jaedicke et al. 2012), a role for a PLA in modulating phyA signalling appears possible.

Coiling

Root coiling in the ppla-I-1 mutant is likely to be caused by decreased gravisensing in the roots, which is a phenotype similar to the phenotype of several auxin-signalling mutants (Simmons et al. 1995; Sedbrook & Kaloriti 2008). Further factors contributing to root coiling are circumnutation and negative thigmotropism (Mullen et al. 1998; Migliaccio & Piconese 2001). Lack of phyA or phyB is known to decrease hypocotyl gravitropism (Robson & Smith 1996) and root gravitropism (Kunihiro et al. 2011), which explains why the phyB mutant strongly and the phyA mutant to a weaker degree produce root coils (Fig. 7). Measurements on the influence of light on auxin transport showed that in tomatoes, phyB2 exerts the strongest positive influence (Liu et al. 2011), supporting our observations that root coils in ppla-I-1 could be a consequence of reduced auxin transport because of compromised phyB signalling in the roots. Because the additional phyD deletion in ppla-I-1 may make it a weak phyB/phyD-like mutant the higher numbers of coils in pplaI-1 as compared with ppla-I-3 could be a result of the missing phyD.

ppla-I mutants are hypersensitive to shade conditions

The most surprising aspect of the ppla-I mutant phenotype was the hypersensitive response to shade light. At the same time, this involvement of pPLA-I in the regulatory interaction of auxin and red light signalling, suggests this is starting with ABP1 as the receptor. The very same phenotypes in abp1–5 and abp1/ABP1, and in ppla-I, together with all the other facets of auxin- and red light-related phenotype elements, provide this hypothesis (Scherer et al. 2012). Potential interactors of phototropism and gravitropism and red light were discussed earlier and may in part explain delayed tropistic responses.

Elongation of hypocotyls in shade is considered to be due to the down-regulation of phyB signal output as the major receptor for this response (Lau & Deng 2010; Stamm & Kumar 2010). Shade avoidance is somewhat enhanced in a phyBphyD double mutant, but also in a phyD single mutant (Devlin et al. 1999), showing that the two genes redundantly regulate this response. phyA has been shown to inhibit hypocotyl extension in light-grown plants and hence, contributes to plant reactions to changes in the R:FR ratio (Johnson et al. 1994). In low R:FR, the phyA mutant has long hypocotyls similar to those of phyB seedlings in high R:FR (Fig. 9). This reaction of phyA seedlings reflects the missing suppression of phyA on hypocotyl elonation in low R:FR shade (i.e.

high FR) conditions in addition to low phyB signalling output. This indeed was also apparent in the mutant lines, and could be complemented by the expression of pPLA-I:GFP (Fig. 9c,d).
A HIR-insensitive or B-insensitive phenotype, as seen in phyA mutants, was not observed (Supporting Information Fig. S4). Only in a combination of FR and B, a hypocotyl growth inhibition was observed (Fig. 9b) supporting our interpretation along the classical views that shade-induced elongation is mainly due to decreased phyB output. The early-flowering phenotype of pplaI-1 could be a result of a decreased output of phyB-like phytochromes in Ws because Ws is a phyD null mutant (Fig. 8a) (Devlin et al. 1999).

Nevertheless, in view of some phyA-influenced phenotypes in the ppla-I mutants, a contribution of phyA-dependent HIR cannot be completely ruled out. Hypersensitivity to shade conditions may explain why a low JA content in ppla-I-1 and ppla-I-2 has been found (Yang et al. 2007). Both their ppla-I-1 and ppla-I-2 alleles are in the Ws background. Moreover, the accumulation of jasmonate in response to Botrytis was indistinguishable in Ws and mutants so that pPLA-I was concluded to not be the enzyme to release linolenic acid for jasmonate biosynthesis (Yang et al. 2007). Jasmonate is an inhibitor of elongation so that depending on the quality and quantity of light, the jasmonate content of plants defective in phyB signalling could be low (Robson et al. 2010). Consistent with this notion is the observation that the jasmonate receptor mutant coi1–16 shows exaggerated sensitivity of hypocotyl elongation to shade (Robson et al. 2010). On that basis, our observations are consistent with the previous findings on pPLA-I functions in JA responses pplaI-1 (Yang et al. 2007).
The ppla-I and abp1 mutants identify a function at the intersection of auxin and light signalling. There is overall similarity of phenotypes of the abp1/ABP1 and abp1–5 mutant and the ppla-I mutants, which consist of the similar regulatory defects in auxin-dependent responses like gene regulation, phototropism and gravitropism (Effendi & Scherer 2011; Effendi et al. 2011, 2013). Additionally, both ppla-I-1 and abp1/ABP1 flower early. As a hypothetical mechanism, we suggested that ABP1 binds to a transmembrane protein as a co-receptor, likely a receptor kinase, which could transmit the auxin signal to a cytsolic network one component of which is suggested to be pPLA-I Effendi et al., 2013). Recent publications described a mechanism to down-regulate phyB and phyB by phosphorylation in the cytosol (Medzihradszky et al. 2013; Nito et al. 2013). There is only one report that the fatty acid oleic acid activates a protein phosphatase and pPLA-I could provide such a metabolite (Baudouin et al. 1999). The results presented here are a platform for further research on this first PLA identified to have functions in light signalling. We therefore conclude that pPLA-I functions downstream of ABP1 and at so far unknown factors common to phytochromes and ABP1.

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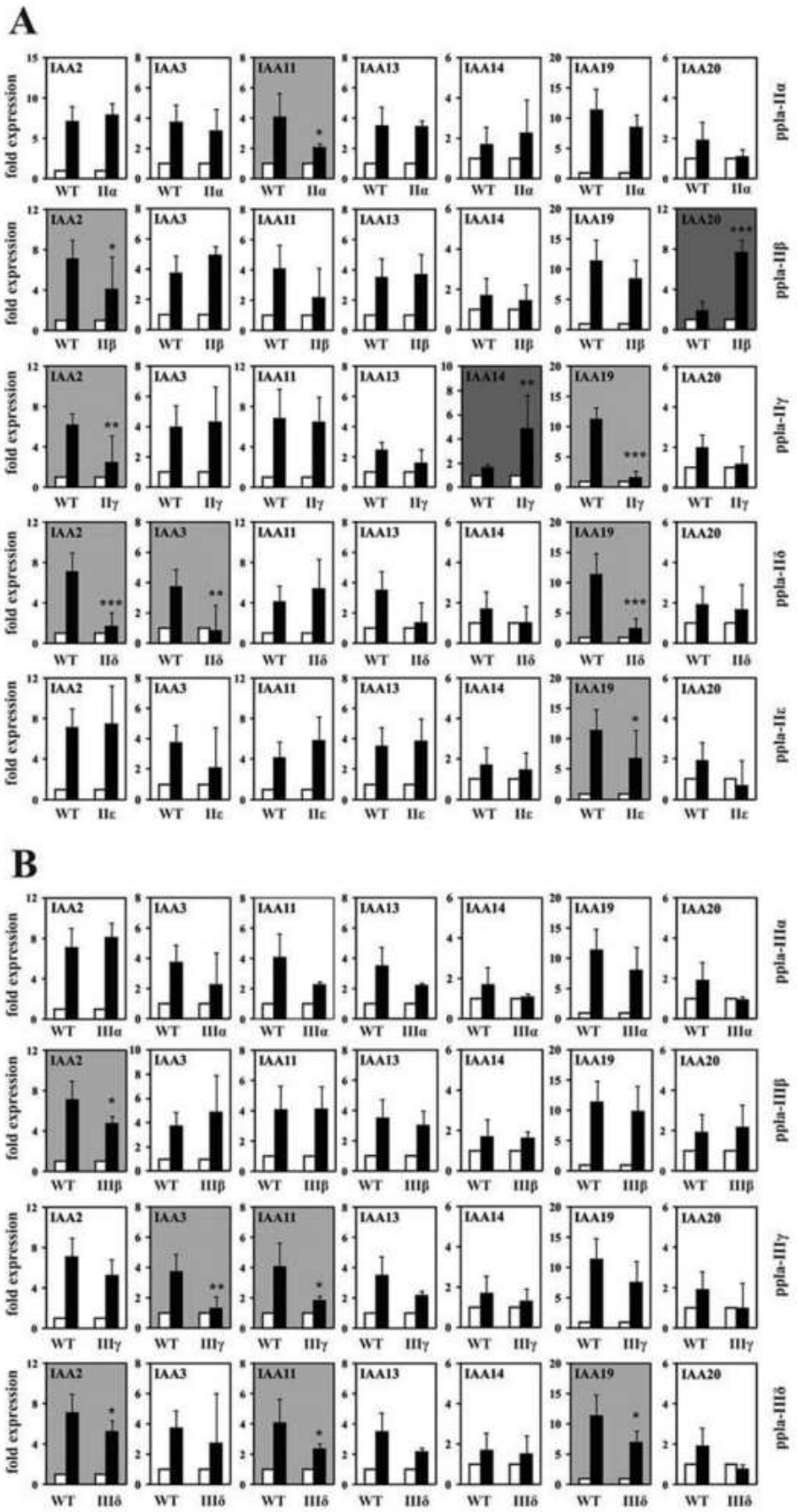
A
B
IAA2
IAA3
IAA11
IAA13
IAA14
IAA19
IAA20
fold expression
WT
IIα
IIβ
IIγ
IIδ
IIε
IIIα
IIIβ
IIIγ
IIIδ
ppla-IIα
ppla-IIβ
ppla-IIγ
ppla-IIδ
ppla-IIε
ppla-IIIα
ppla-IIIβ
ppla-IIIγ
ppla-IIIδ

Figure 1. Expression of IAA Genes in Light-Grown ppla Mutant and Wild-Type Seedlings. (A) Group II genes. (B) Group III genes. The background of the panels is shaded whenever significant differences between wild-type and mutant were obtained

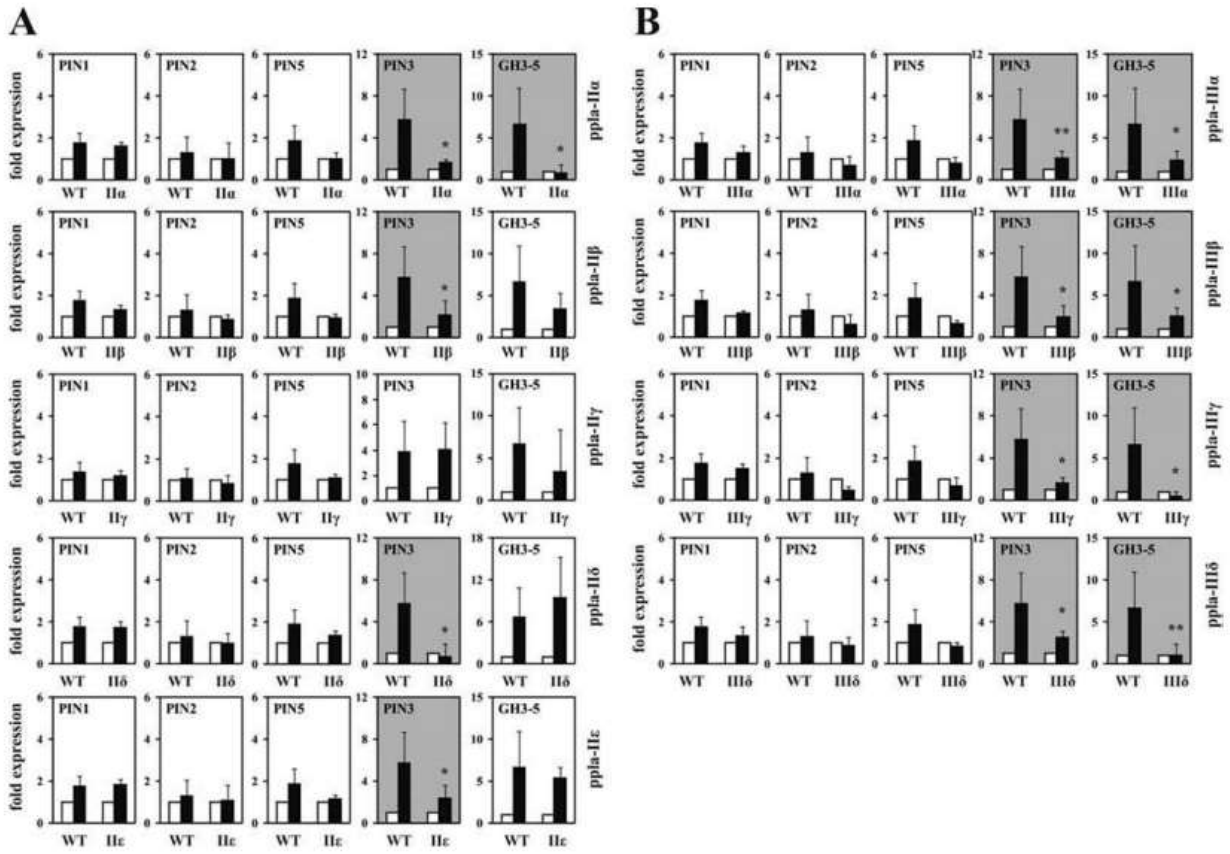

Figure 2. Expression of Several PIN Genes and GH3.5 in Light-Grown ppla Mutant and Wild-Type Seedlings. (A) Group II genes. (B) Group III genes. The background of the panels is shaded whenever significant differences between wild-type and mutant were obtained.

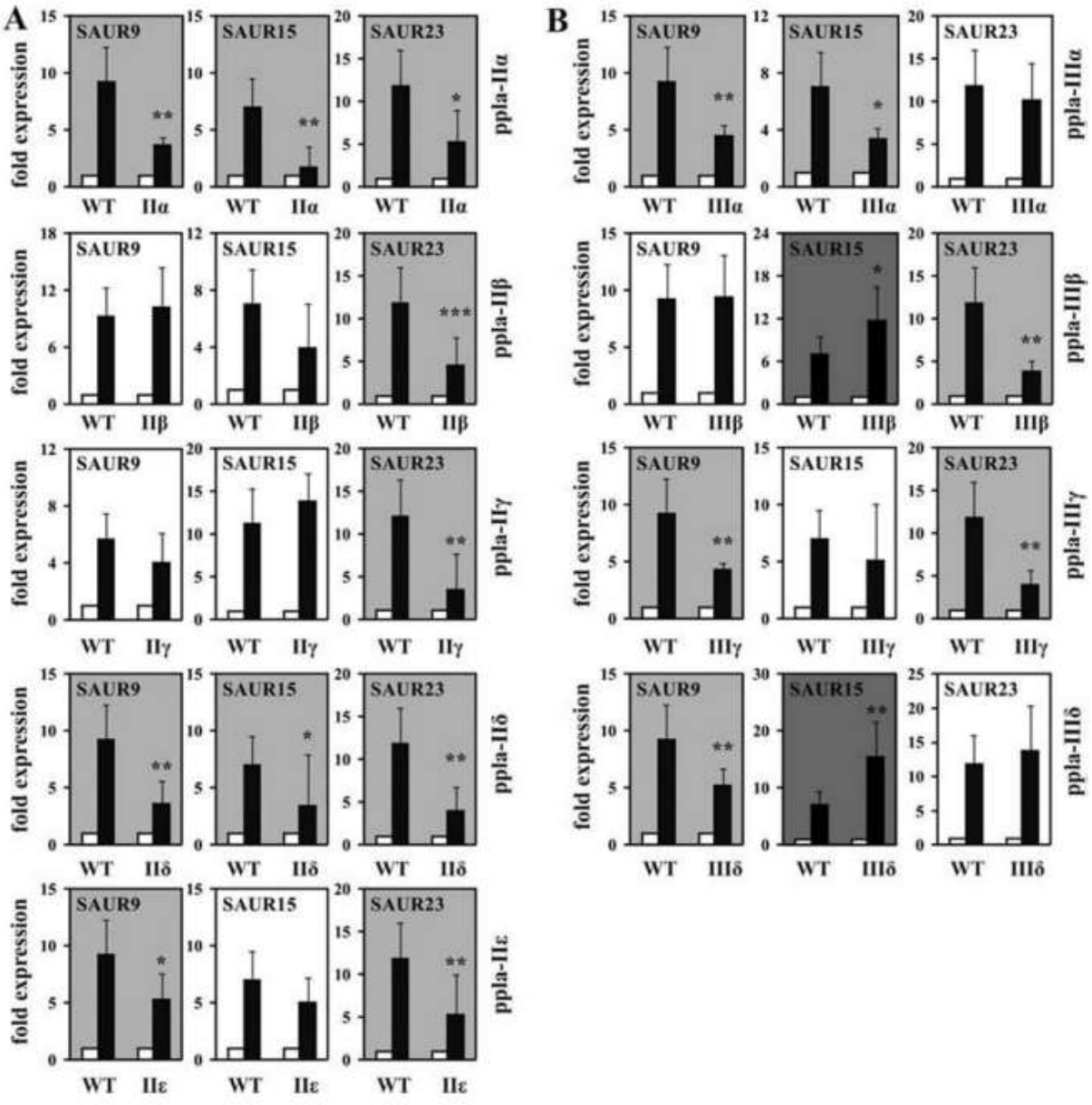

Figure 3. Expression of Several SAUR Genes in Light-Grown ppla Mutants and Wild-Type Seedlings Grown in the Light. (A) Group II genes. (B) Group III genes. Background of panels is shaded whenever significant differences between wild-type and mutant were obtained.

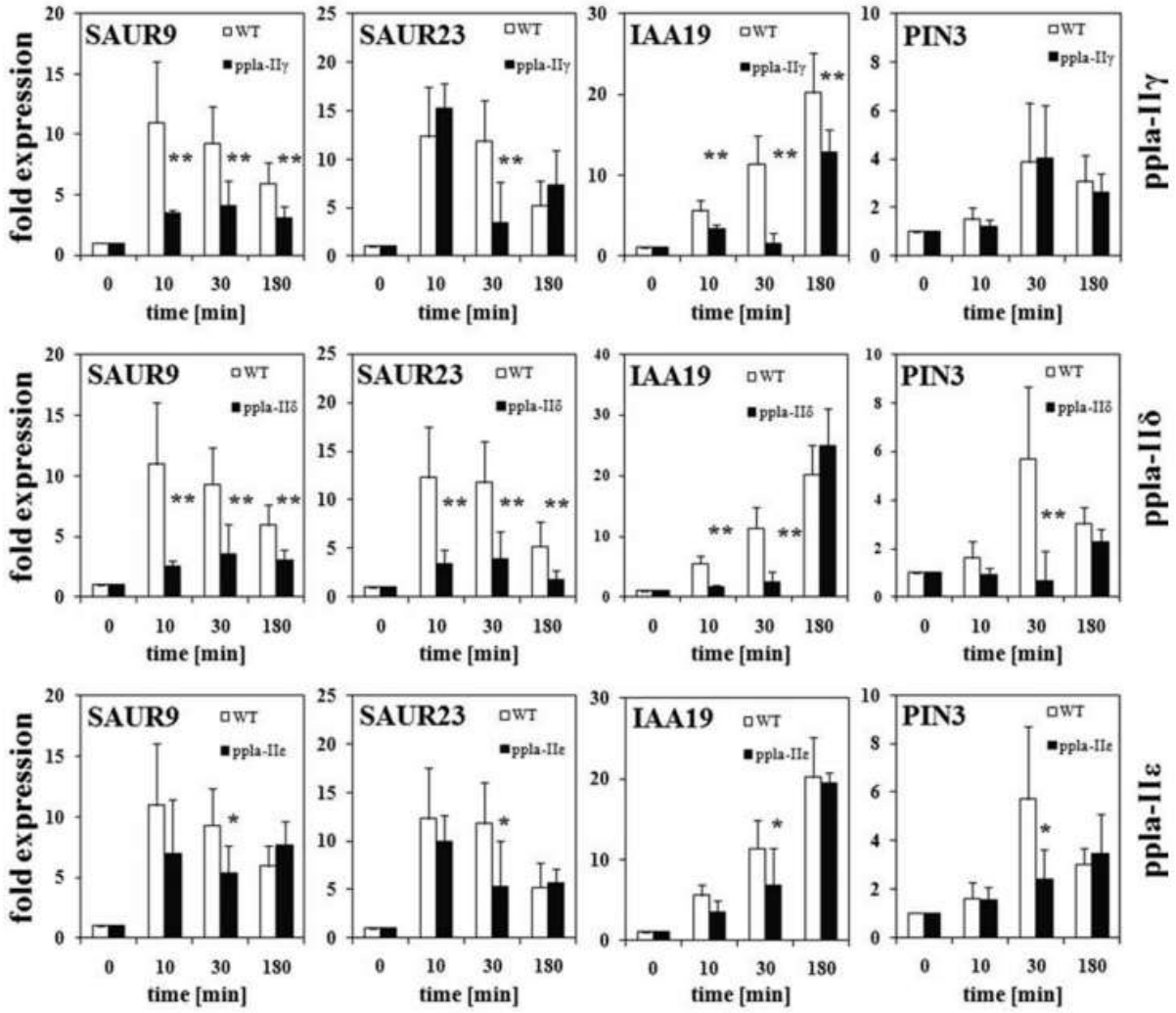

Figure 4. Time Courses of Expression of Selected Genes in Three Light-Grown ppla Mutant and Wild-Type Seedlings.

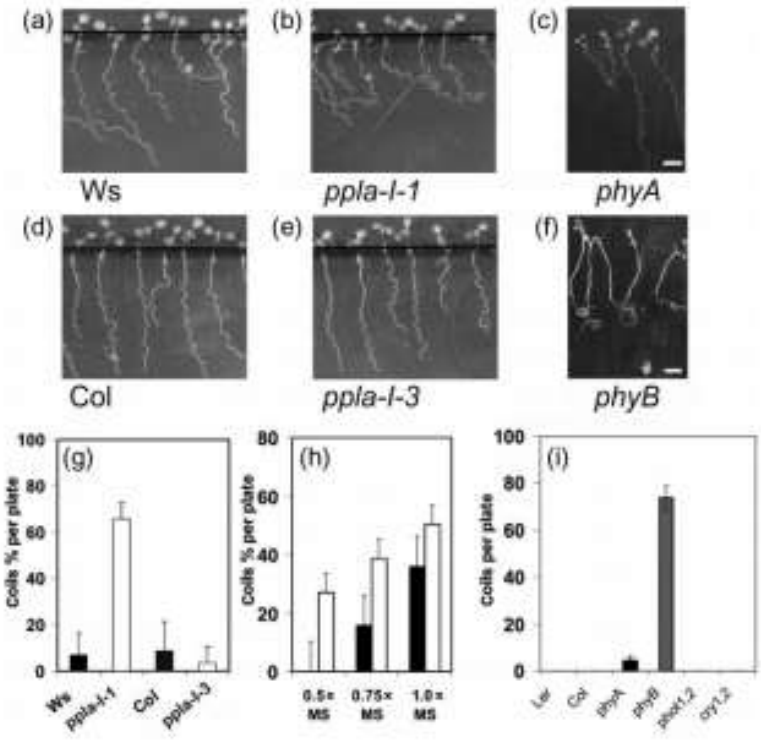

Figure 5. Formation root coils in seedlings grown on hard agar at a 45° tilted angle in (a,b,d,e) on 1/1 MS agar and in (c, f) on one-half MS agar. A plate with Ws wild type (WT) (a), pplaI-1 (b), phyA (c), Col (d), ppla-I-3 (e), and phyB in (f) is shown. (g) Comparison of coil frequency on 1/1 MS medium of the two mutant ppla-I lines. (h) Dependence of coiling response on the osmolarity of the medium in ppla-I-1 (white bars) and Ws WT (black bars). (i) Comparison of coiling in of several photoreceptor mutants on one-half MS agar. Each experiment was conducted with four to six agar plates harbouring 15 or 30 seedlings each (SE)

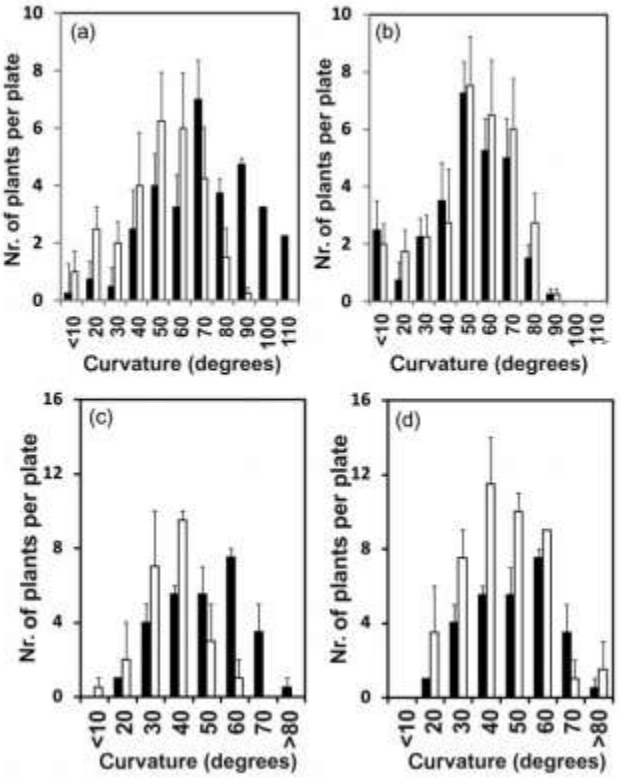

Figure 6. Phototropic bending of hypocotyls of 3-day-old dark-grown seedlings after 12 h lateral blue light (1 E). Black bars: WT, white bars: mutants. (a) Ws: 67.1°, ppla-I-1: 45.6° ($P < 0.001$). (b) Col: 46.6°, ppla-I-3: 47.2°. (c) Col: 51.2°, phyA: 33.1° ($P < 0.001$). (d) Col: 51.2°, phyB: 41.2° ($P < 0.001$). (means indicated, n = 66–129)

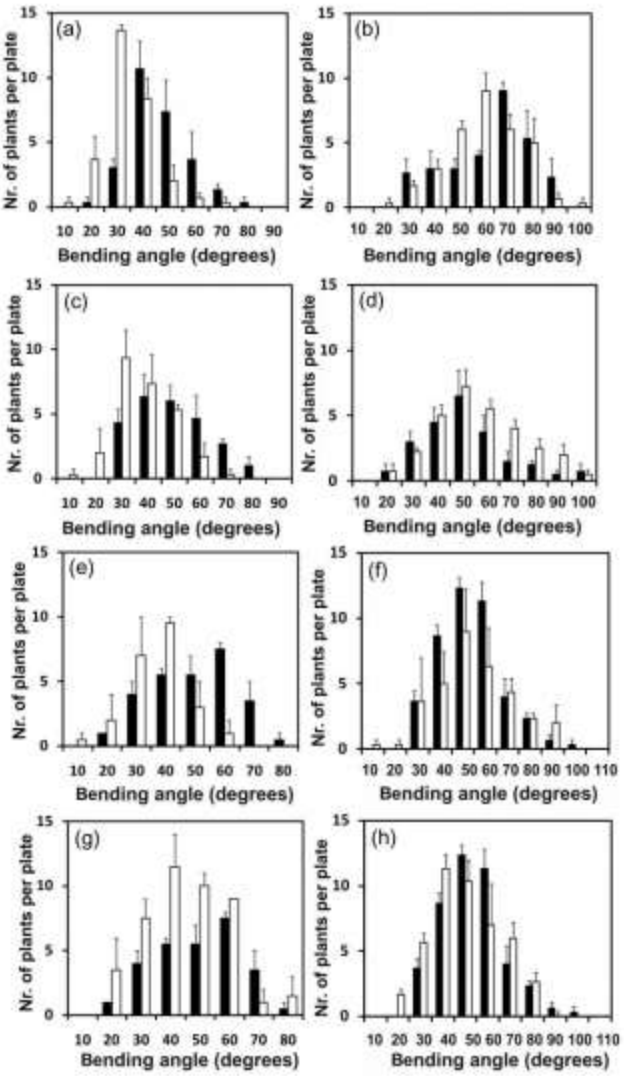

Figure 7. Gravitropic bending of hypocotyls or roots of 3-day-old dark-grown seedlings after 24 h tilting by 90°. (a,c,e,g) Hypocotyl bending angle frequencies. (b,d,f,h) Root bending angle frequencies. Black bars: WT. White bars: mutants. (a) Ws: 43.1°; ppla-I-1: 30.0° ($P < 0.001$). (c) Col: 45°; ppla-I-3: 33.8° ($P < 0.001$). (e) Col: 51.6°; phyA: 48.9°. (g) Col: 51.6°; phyB: 40.4° ($P < 0.001$). (b) Ws: 58.5°; ppla-I-1: 54.7°. (d) Col: 46.4°; ppla-I-3: 52.4° ($P < 0.014$). (f) Col: 54°; phyA: 54.9°. (h) Col: 54° phyB: 51.1°. Means indicated, n = 73–120.

PATATIN-RELATED PHOSPHOLIPASE A KNOCKOUT MUTANTS HAVE DEFECTS IN REGULATION OF EARLY AUXIN-INDUCED GENES

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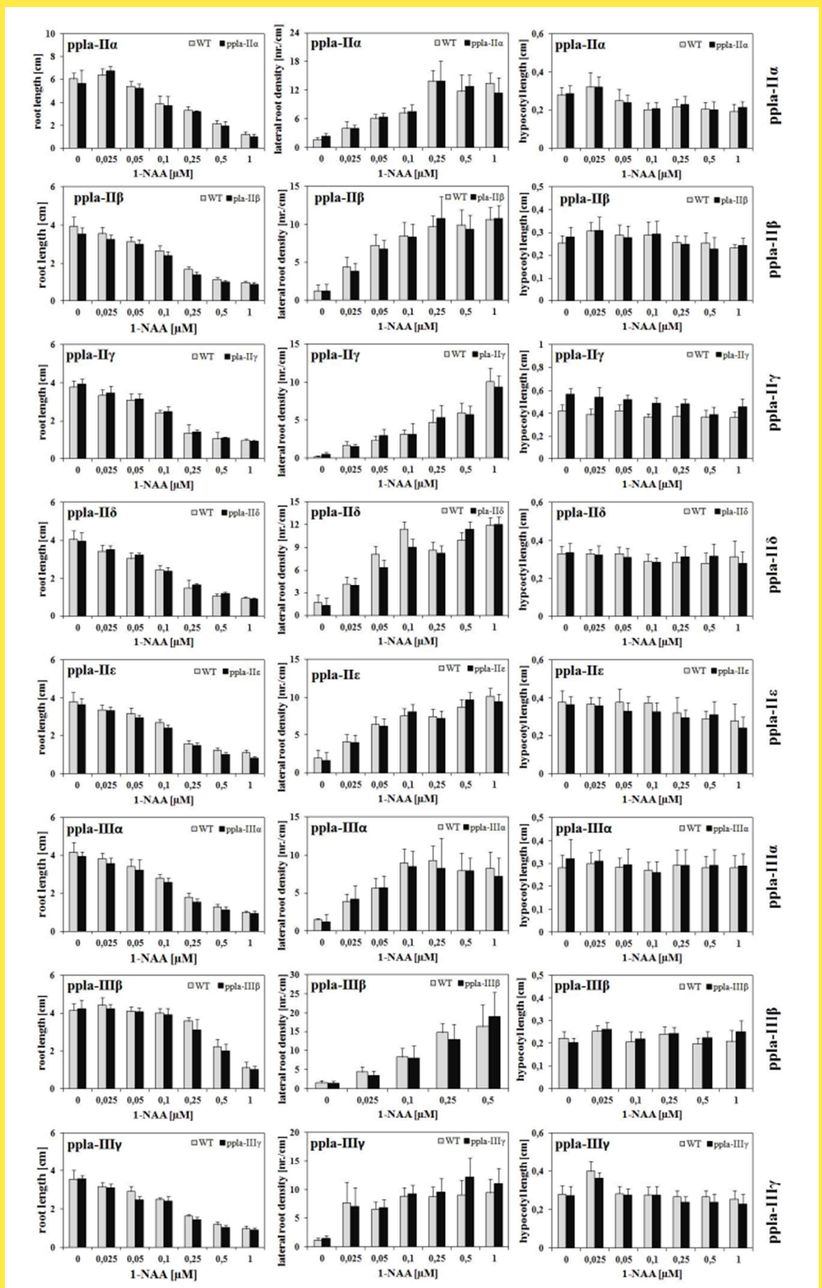
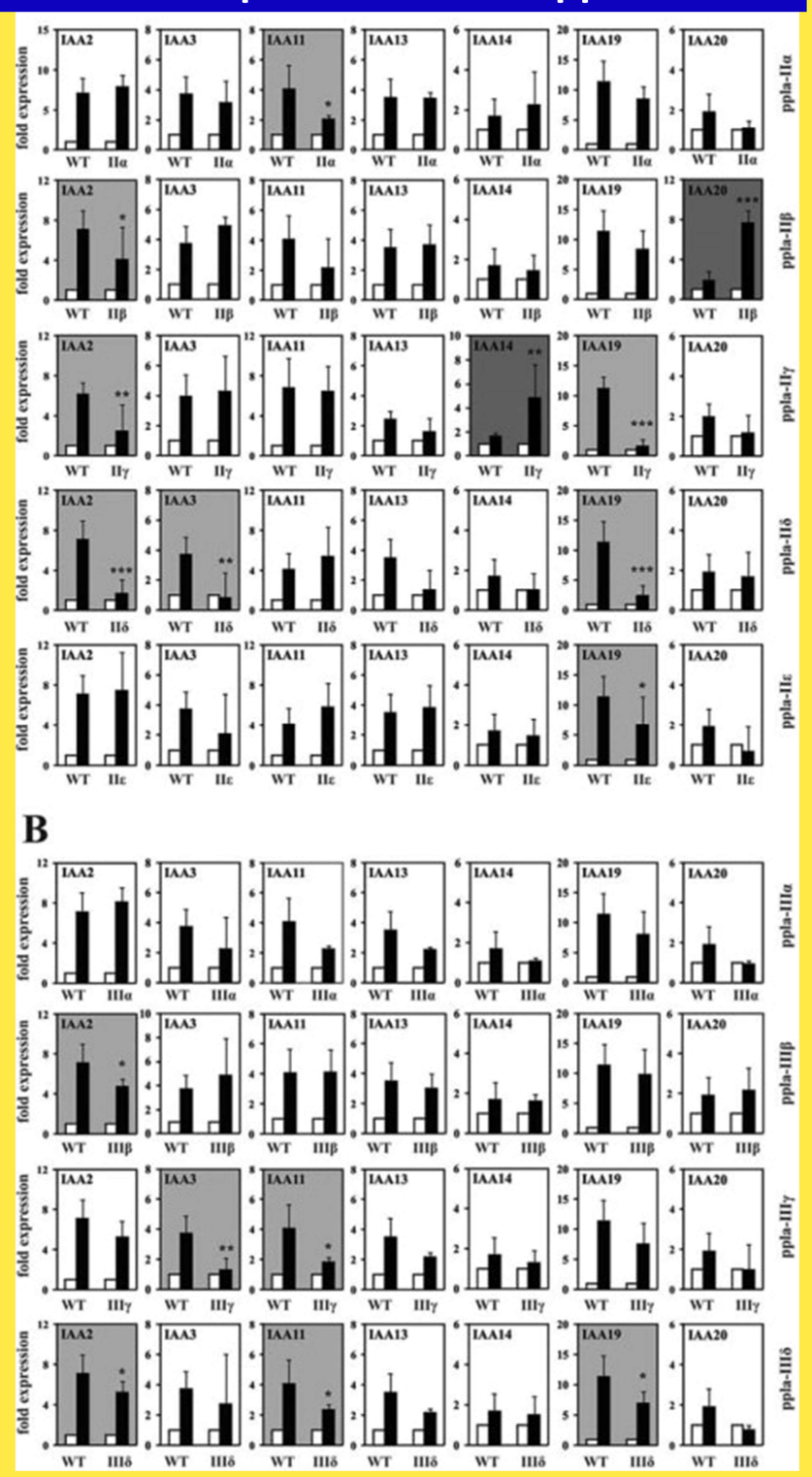
In Arabidopsis, a family of ten phospholipase A genes has been identified and are involved in auxin and pathogen signaling (Rietz et al, 2010, Mol. Plant). Plant PLA activity is rapidly induced by different external signals an the PLA reaction products function as secondary messengers in plant signal transduction (Scherer et al, 2010, TIPS). Here we used the knockout mutants of all ten pPLAs to test the regulation of early auxin genes. Test genes were IAA-genes, SAUR-genes, genes involved in lateral root formation (Perez et al, 2009) and PIN-genes. Many of the lateral root genes and the SAUR genes showed a strong defect in genes expression in the pPLA knockouts after 10uM auxin application (t=30 min), in comparison, the transcription of pPLA genes themselves is not auxin regulated within 30 min. The pPLA knockouts did not show any phenotypes under normal growth conditions or when grown on auxin containing medium. In summary, the pPLA knockouts show a transient mis-regulation of early auxin regulated genes that mostly dissapeared after 3 hours. Because the abp1/ABP1 mutant regulated of none early auxin-induced genes at 30 min we hypothezise that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi et al, 2011, Plant J.)

Physiological respond of eight T-DNA insertion *pplA* mutants in response to auxin application



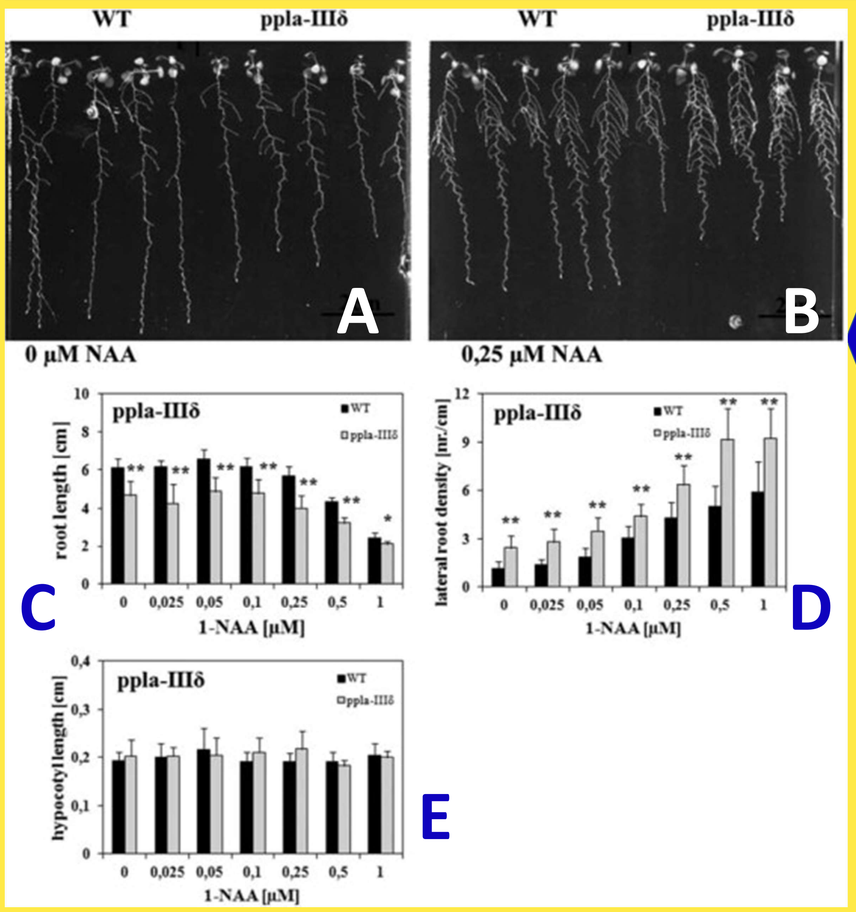
Physiological responses to auxin of T-DNA insertion mutants in root dan hypocotyl length, and lateral root density. Plants were grown in white light for 7 d on upright agar plates in the presence of increasing concentrations of 1-NAA. None of *ppla* mutants showed an auxin phenotype, except *ppla-IIIδ* mutants

Level expression of IAA genes in *pplA* mutants in response to auxin application.



Expression of *IAA* Genes in Light-Grown *ppla* Mutant and Wild-Type Seedlings. The background of the panels is shaded whenever significant differences between wild-type and mutant were obtained. Asterisks above columns indicate significant differences between the mutants and the corresponding wild-type treatments type (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *t*-test). Relative expression levels were calculated by setting values at t = 0min to 1 (white bars); values at t = 30 min IAA were calculated accordingly (black bars).

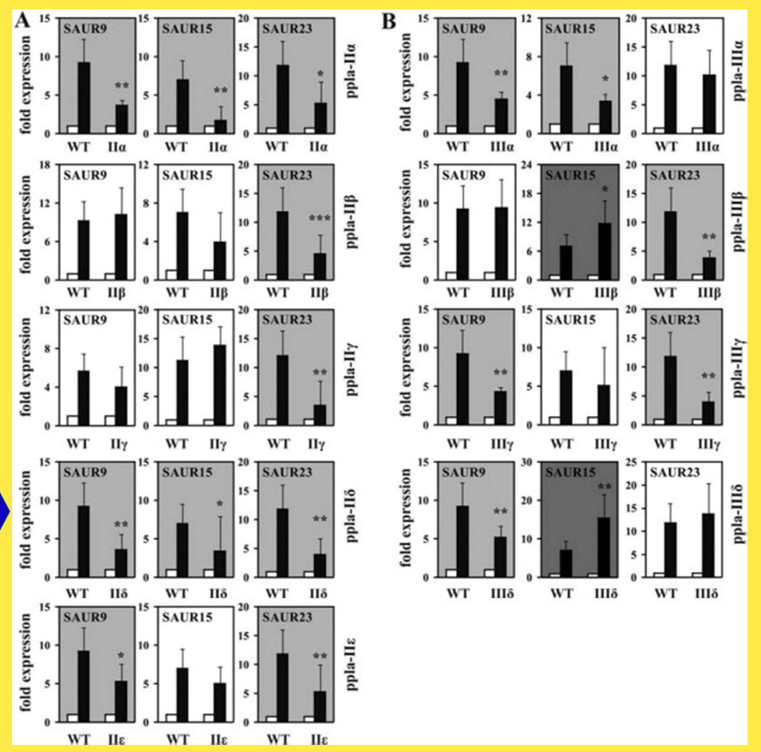
Growth Response of Light-Grown *ppla-IIIδ* Mutants and Wild-Type Plants in Response to Auxin.



Seedlings were grown for 7 d on 1 ATS medium with different 1-NAA concentrations. **(A)** Comparison of growth patterns (bar = 2 cm). **(B)** Root length. **(C)** Lateral root density. **(D)** Hypocotyl length. Asterisks above columns indicate significant differences between treatments of mutant and the corresponding wild-type (* $p < 0.05$; ** $p < 0.01$; *** $p < 0,001$; *t*-test).

All three *SAURs* (*SAUR9, SAUR15, SAUR23*) were less up-regulated. *ppla-IIIβ* and *ppla-IIIδ* were unusual in that, here, *SAUR15* responded more strongly when compared to the wild-type. **(A)** Group II genes. **(B)** Group III genes. Background of panels is shaded whenever significant differences between wild-type and mutant were obtained.

Expression of Several *SAUR* Genes in Light-Grown *ppla* Mutants and Wild-Type Seedlings Grown in the Light.



Conclusion

The pPLA knockouts show a transient mis-regulation of early auxin regulated genes that mostly dissapeared after 3 hours. Because the abp1/ABP1 mutant regulated of none early auxin-induced genes at 30 min we hypothezise that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (Effendi et al, 2011, Plant J.)