Black Forest Summer School 2013
Bioinformatics for Molecular Biologists
Herzogenhorn, Sep 10th - 13th
http://plantco.de/BFSS2013/

Letter of Confirmation

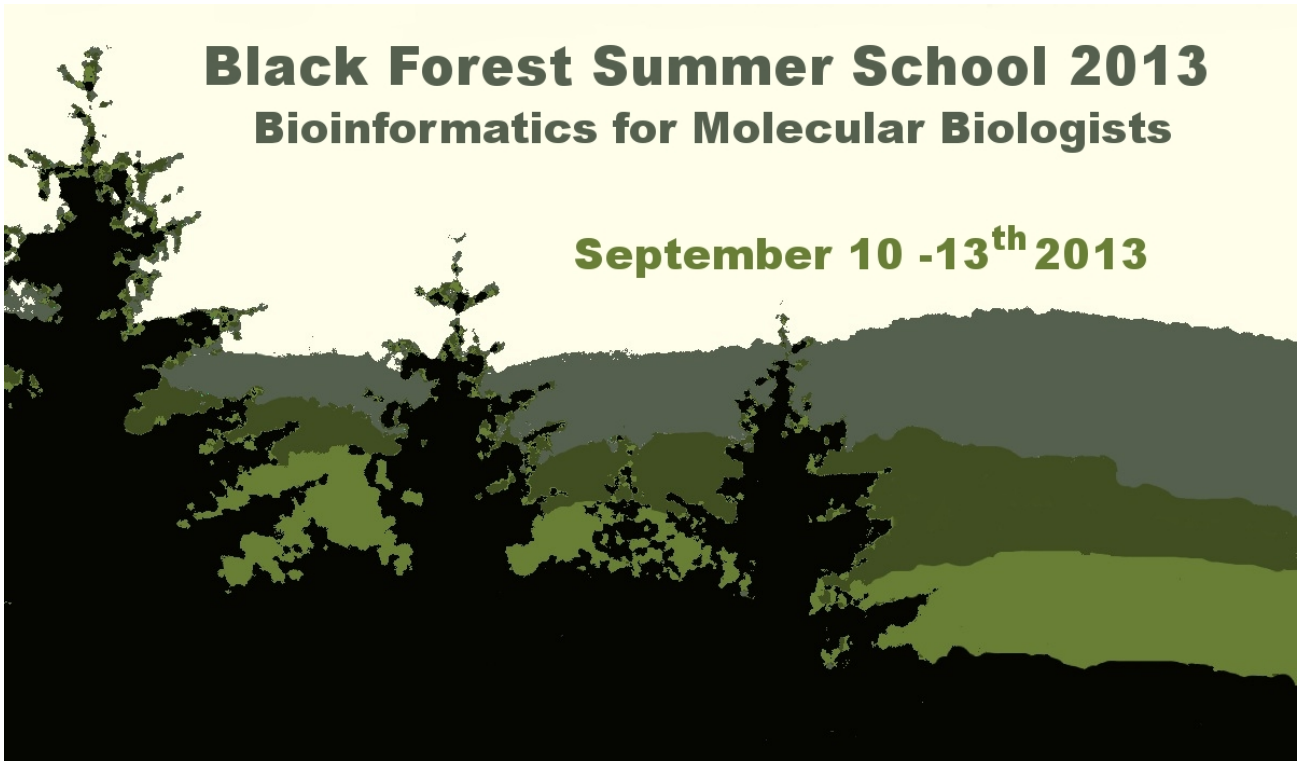
This is to confirm that

Yunus Effendi

attended the international 'Black Forest Summer School 2013' from September 10th to September 13th, 2013 in Herzogenhorn, Black Forest, Germany.

Stefan A. Rensing, PhD
(Organizer)

The Black Forest Summer School 2013 on Bioinformatics for Molecular Biologists is organized by Plantco.de e.V. (Freiburg, Germany) and supported by the Freiburg Institute for Advanced Studies (FRIAS). Support by the companies shown below is gratefully acknowledged.



September 10th - 13th 2013

http://plantco.de/BFSS2013

Abstract Book

Edited by Stefan A. Rensing and Kristian Ullrich
Marburg, Germany, September 2013

Venue: Leistungszentrum Herzogenhorn (Black Forest Highlands, Germany)
~1,300 mtrs above sea level

Acknowledgements

The Black Forest Summer School 2013 on Bioinformatics for Molecular Biologists is organized by Plantco.de e.V. (Freiburg, Germany) and supported by the Freiburg Institute for Advanced Studies (FRIAS) and the German Botanical Society (DBG). Additional support by the companies shown below is gratefully acknowledged.

Thank you very much!

Contents

Program

Venue: Leistungszentrum Herzogenhorn

All workshops and talks take place in the seminar room. Poster sessions and industry exhibit take place in the gymnasium. Breakfast, lunch, supper, coffee breaks and evening entertainment are located in the dining rooms.

Tuesday Sep 10th

15:00 - 17:30	Bus shuttles from Feldberg-Bärental train station to venue (round-trip ~20min)
15:15 - 19:00	Registration desk open *(also open during the conference at all breaks)*
19:00 - 20:00	**Reception with food and beverages**
20:00	**Welcome, organizational remarks**
20:15	**Keynote lecture: Francis Martin** (INRA Nancy, France) **The forest social network:** **Deciphering the molecular language of mycorrhizal symbiosis**
later	*"moss cocktail workshop"*

Wednesday Sep 11th

7:45 - 8:45	Breakfast
9:00 – 10:45	**Workshop 1: *Stefan Rensing*** (University of Marburg, Germany) **Things you always wanted to know about BLAST and never dared to ask** **+ How do I do phylogenies?!**
10:45	Coffee break
11:00 – 11:30	**Workshop 1 *continued***
11:30 – 12:00	**Oral session I (contributed talks)**

11:30 T1 *Jose Sergio Hleap* (Dalhousie University, Halifax, Canada)
Defining Structural and Evolutionary Modules in Proteins:
A Community Detection Approach to explore sub-domain architecture

11:45 T2 *Natalie Laibach* (Fraunhofer Institute for Molecular Biology and Applied Ecology, Münster, Germany)
Small Rubber Particle Proteins facilitate rubber biosynthesis and play a role in abiotic stress response

12:00	Lunch
13:00 – 14:00	**Poster session I with coffee** ***ODD* numbered posters**
14:00 – 15:00	**Workshop 2: Anne Hamm** (Roche, Germany) **qPCR setup and analyses**

Excursion	
15:15	*Departure*
15:30	*Uphill drive with Seebuck cable car*
16:00	*Reception on 11th floor of Feldberg tower*
16:45	*Glacial relict excursion (Michael Scherer-Lorenzen, Unversity of Freiburg, Germany) and walk down*
~18:00	*Return*
18:30	Supper
later	*"Casino Royale"*

Thursday Sep 12th

7:45 - 8:45	Breakfast
9:00 – 10:45	**Workshop 3: Emily Pritchard** (EBI Hinxton, UK) **Ensembl / Ensembl Genomes for studying animal and fungal genomes**
10:45	Coffee break & industry exhibits (poster hall)
11:15 – 12:00	**Workshop 3 *continued***
12:00	Lunch (& industry exhibits)
13:00 – 14:00	**Poster session II with coffee & industry exhibits** ***EVEN* numbered posters**

14:00 – 15:00 **Poster session III & industry exhibits**
Discussion of *ALL* posters

15:00 – 17:00 **Workshop 4: Sebastian Proost** (MPI Golm, Germany)
PLAZA for plant comparative genomics

17:00 – 17:30 **Oral session II (contributed talks)**

17:00 T3 *Sabine Fischer* (Johannes Gutenberg-Universität, Mainz, Germany)
Next-generation sequencing of pooled DNA samples uncovers genome-wide footprints of selection in wild grapevine

17:15 T4 *Elisabet Ottosson* (Swedish University of Agricultural Sciences, Uppsala, Sweden)
Deep sequencing of decomposing wood reveals the diverse ecological roles within fungal communities in logs

18:00 Supper

later *Farewell party*

Friday Sep 13th

7:45 - 8:45 Breakfast

9:00 – 10:45 **Workshop 5: Rensing lab and Björn Grüning**
(University of Marburg/Freiburg, Germany)
Next generation DNA sequencing and analysis of NGS data

10:45 Coffee break

11:00 – 11:45 **Workshop 6: Arnd Brandenburg** (Genedata, Basel, Switzerland)
Large scale multivariate data analyses

11:45 **Concluding remarks, best poster and best talk reward**

12:00 Lunch, end of retreat

13:30, 14:30 Bus shuttles to Feldberg-Bärental train station

Poster Abstracts

P1 Low degree of host specificity among arctic ectomycorrhizal fungi

Synnove Botnen[1,2], Unni Vik[1], Tor Carlsen[1], Pernille Bronken Eidesen[2], Marie Davey[2], Håvard Kauserud[1]

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In arctic ecosystems, low soil moisture and nutrient availability, low soil and air temperatures, as well as a short growing season limits plant growth and reproduction. Mycorrhiza facilitates plants nutrient acquisition and water uptake, and may therefore be particular important in nutrition poor and dry environments like the Arctic. However, little is known about the host specificity of arctic mycorrhizal fungi.
Bistorta vivipara, *Salix polaris* and *Dryas octopetala* are keystone arctic plant species, all forming ectomycorrhiza with a wide range of fungi. The host specificity of the root-associated fungal communities in these three plants were investigated using high throughput sequencing of the internal transcribed spacer 1 (ITS1) amplified from whole root systems of sixty plants collected in the High Arctic archipelago Svalbard. No sign of host specificity was found, and no spatial autocorrelation was observed within two 3 m x 3 m sample plots. Moreover, no significant differences in fungal richness were observed across the three plant species. The roots were dominated by the ectomycorrhizal basidiomycote orders Agaricales, Sebacinales and Thelephorales. In the Arctic, it may be of pivotal importance for the fungi to be able to colonize the closest potential plant host, as the growing season is short and the vegetation is scarce. The lack of spatial structure at small spatial scales further suggests that common mycelial networks are rare in the marginal arctic environments.

P2 New abp1 mutants show impairment of auxin-related functions and defect in red and far red light responses

Yunus Effendi, Markus Geissler, Guenther F.E. Scherer

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Auxin Binding Protein1 (ABP1) has been proposed as membrane-bound auxin receptor in plants based on early studies. Recent progress in ABP1 research suggested that ABP1 could be auxin receptor for rapid auxin-related processes.
We designed and characterized four in-vitro abp1 mutants containing point mutation in the presumed auxin binding site of ABP1. The abp1 mutants showed defects in auxin-related functions such as in gravitropic and phototropic (root and shoot) responses, early flowering, insensitivity to auxin and reduced transcript levels of early auxin responsive genes (AUX/IAAs, GH3, SAURs) and auxin efflux transporter genes (PINs). Additionally, the abp1 mutants exhibited insensitivity of hypocotyl elongation inhibition to red and far-red light and showed hypersensitive hypocotyl elongation to shade light. qPCR data of shade-induced genes in response to FR- and R-enriched white light was altered in all abp1 mutants in compared to WT. This provides initial evidence of a regulatory link between auxin and phyB-mediated light responses via ABP1 action. Taken together, the new abp1 mutants showed mutant properties not only as auxin mutants but also light mutants.

New Auxin Binding Protein1 (ABP1) mutants show impairment of auxin-related functions and defect in red and far red light responses

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Auxin Binding Protein1 (ABP1) has been proposed as membrane-bound auxin receptor in plants based on early studies. Recent progress in ABP1 research suggested that ABP1 could be auxin receptor for rapid auxin related processes. We designed and characterized four in-vitro abp1 mutants containing point mutation in the presumed auxin binding site of ABP1. The abp1 mutants showed defects in auxin-related functions such as in gravitropic and phototropic (root and shoot) responses, early flowering, insensitivity to auxin and reduced transcript levels of early auxin responsive genes (AUX/IAAs, GH3, SAURs) and auxin efflux transporter genes (PINs). Additionally, the abp1 mutants exhibited insensitivity of hypocotyl elongation inhibition to red and far-red light and showed hypersensitive hypocotyl elongation to shade light. qPCR data of shade induced genes in response to FR-and R-enriched white light was altered in all abp1 mutants in compared to WT. This provides initial evidence of a regulatory link between auxin and phyB-mediated light responses via ABP1 action. Taken together, the new abp1mutants showed mutant properties not only as auxin mutants but also as light mutants

Keyword: ABP1, early auxin-induced genes, phototropism, phytochrome B

Introduction

Auxin is a phytohormone that it is known to regulate many physiological processes of growth and development in plants such as morphogenesis, organogenesis, reproduction, secondary growth, apical dominance, cell elongation and division, and tropic response of root and shoot in response to external stimuli such as light and gravity (Davies 1995; Leyser, 2006; Benjamins and Scheres, 2008; Mockaitis and Estelle, 2008; Chapman and Estelle, 2009). At the molecular level, many genes are known to be regulated by auxin (Hagen and Guilfoyle, 1985; McClure and Guilfoyle, 1987; Abel and Theologis, 1996; Remington et al., 2004; Okushima et al., 2005; Overvoorde et al., 2005).

A conditional ABP1 mutant was created by expressing an antibody against ABP1 in the apoplast which suppressed ABP1 functions like leaf expansion, endomitosis, cell division, and cell expansion (David et al., 2007; Braun et al., 2008; Paque et al., 2014), results verified with an inducible mutant (Jones et al., 1998; Chen et al., 2001a). The only known T-DNA insertion mutant of this gene proved to be embryo-lethal (Chen et al., 2001b). The point mutation abp1-5, obtained by TILLING, was useful to uncover the interaction of ABP1, PIN proteins, and ROP/RIC signalling in protein trafficking (Robert et al., 2010; Xu et al., 2010). More detailed investigations using the heterozygous ABP1/abp1 T-DNA insertion line revealed that functions like auxininduced gene expression, phototropism and gravitropism, and auxin transport are defective in this mutant (Effendi et al., 2011; Effendi and Scherer, 2011). Recently ABP1 hasbeen linked to red light physiology, using ABP1/abp1 and abp1-5 (Effendi et al., 2013), and to control of TIR1 activity (Effendi et al., 2011; Tromas et al., 2013).

Both ABP1/abp1 and abp1-5 have weak phenotypes so that progress in ABP1 research based on these mutants is still limited. On the other hand, the embryo lethality of a homozygous T-DNA insertion plant (Chen et al., 2001b) opened up the possibility to complement this plant not only with wildtype but also with

point-mutated cDNAs. We describe here such a series of mutants based on complementation of the knock-out plant that show more severe auxin-related phenotypes than previous abp1 mutants. These results reveal that not only auxin but also phytochrome signaling is compromised in these lines.

The cross-talk between auxin and light in the growth regulation of the plants has been intensively investigated (Behringer and Davies, 1992; de Lucas et al., 2008), especially in response to shade avoidance (Morelli and Ruberti, 2002; Tanaka et al., 2002; Lorrain et al., 2008; Sorin et al., 2009; Kozuka et al., 2010; Keuskamp et al., 2010). Auxin-responsive functions were reported to be affected by light, such as gravitropism (Haga and Iino, 2006), hypocotyl elongation (Steindler, 1999; Tao et al., 2008), petiole elongation (Tao et al., 2008; Kozuka et al., 2010), auxin polar transport (Kanyuka et al., 2003; Keuskamp et al., 2010; Liu et al., 2011) and auxin biosynthesis (Tao et al., 2008). The effect of auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) in the reduction of hypocotyl shade avoidance response was also demonstrated (Steindler et al., 1999; Pierik et al., 2009). Moreover, global expression profiling revealed that some early auxin-responsive genes are also induced by low ratio red:far red (R/FR) (Devlin et al., 2003; Sessa et al., 2005; Tao et al., 2008). This all indicates a close regulatory link between auxin and light responses.

In this study, we designed a new class of *abp1* mutants and examined their functional roles in responses to auxin. Several auxin-induced physiological functions and auxin-responsive transcription were investigated. Furthermore, cross-talk between auxin and light pathway was investigated in the *abp1* mutants by characterizing their auxin-mediated responses as well as transcriptional levels of light-responsive genes. Here, we show that *abp1* mutants have defects in auxin-related physiological functions such as root and hypocotyl gravitropic response, phototropic response, sensitivity to auxin of root growth and a lower transcription level in auxin-responsive genes. We also observed that a defect had lead to insensitivity in red light responses as well as a hypersensitive shade avoidance response as a consequence. Furthermore, alteration in expression of light-induced genes induced by far red or red addedto white light in the *abp1* mutants was observed. Thus, taken together this study provides evidence that *ABP1* is necessary for crosstalk of auxin and phytochrome signaling.

RESULTS

Auxin-related functions in *abp1* mutants are defect

We designed and developed new *abp1* mutants of *Arabidopsis* by transforming *ABP1* cDNA containing mutations in the putative auxin binding sites of ABP1 (Woo et al., 2002) into heterozygous T-DNA insertion *abp1/+* mutant (Chen et al., 2001).

We wanted to eliminate wild type ABP1 protein from these plants by selecting lines homozygous for the insertion so that effects of loss of function in viable plants might be observed. However, it previously has been reported by Chen *et al.* (2001) homozygous null *ABP1* mutant plants are lethal in the embryo stage, thus complete null *ABP1* plants were never present. The expression of the recombinant ABP1 cDNA was under control of 35S promoter. At C-terminal position, a strep II tag and a flag tag were inserted before the C- terminal KDEL.

Through double selection of transformed progeny on BASTA and kanamycin containing agar, followed by PCR genotyping with primers against the insertion allele of *ABP1* (Chen et al., 2001), we were able to isolate three stabile *abp11* mutants, *abp1-8 (*Thr54>Ile54), *abp1-9* (Leu25>Tyr25) and *abp1-10* (His106>Asn106).

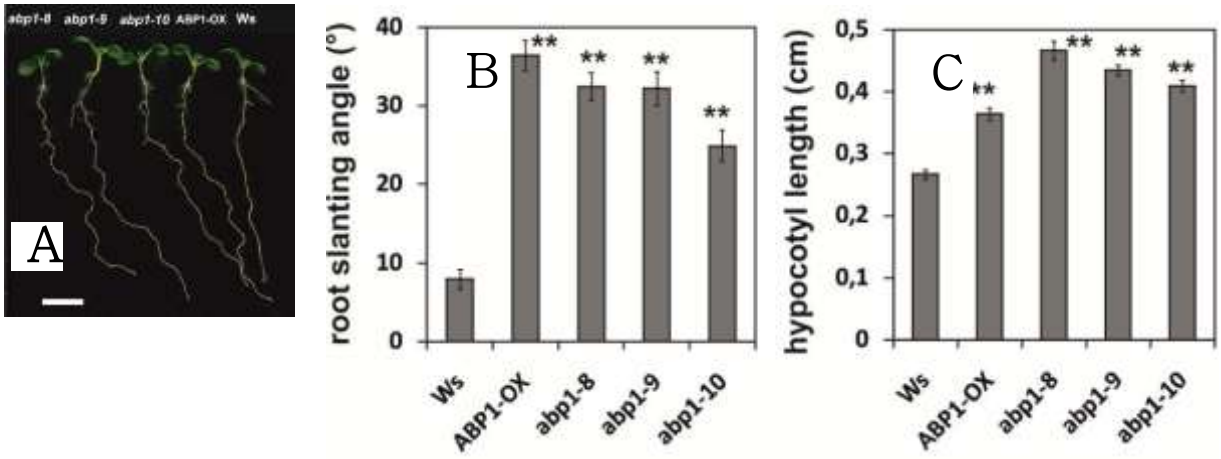

Figure 1. Slanting and waving of roots and elongated hypocotyls in *abp1* mutants. (A) Representative images of 7 days light grown seedlings Ws, *ABP1-OX* and *abp1* mutants. Scale bar = 0.5 cm (B) Seedlings were grown on ½ MS media containing 1% sucrose and 0.5% Gelrite (Duchofa-Biochemie). After 7 days growth under 8h/16h white light condition, slanting angles were quantified. (C) Hypocotyl length of 10 days light growth seedlings. For both experiments, data were collected from three independent experiments, each replication contains n > 25 seedlings for each lines. Values are means ± S.E. ($p < 0.001$ for in-vitro *abp1* mutants versus Ws). Bar = 5 mm.

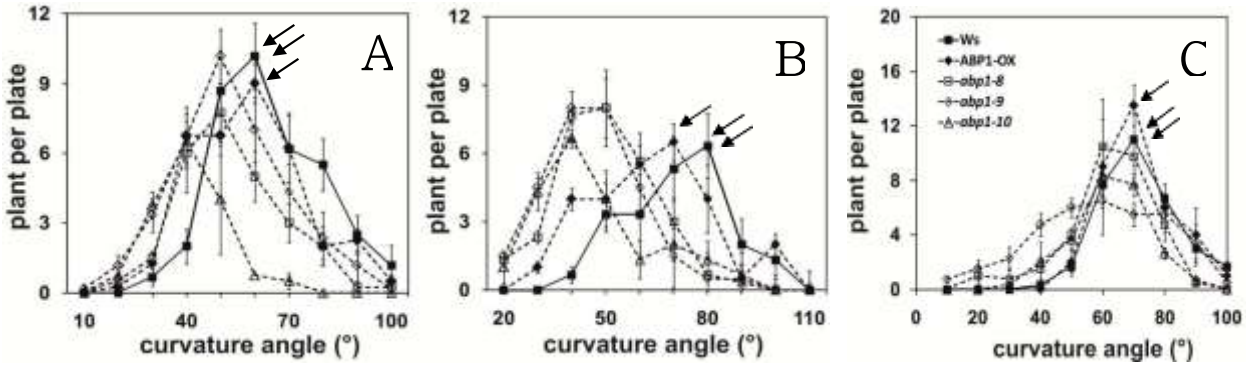

Figure 2. Response to gravity and lateral blue light in Ws, *ABP1-OX* and *abp1* mutants.

Gravitropic responses of the hypocotyls of 3 days old dark-grown seedlings. Seedlings were grown vertically for 4 days in the dark, tilted by 90° for 24 hours and gravitropic angles were determined. Data were obtained from five plates for each genotype with n > 150 total of seedlings. Data are means (± S.E.) of seedling per plate (B) Gravitropic responses of the roots of 3 days-old dark-grown seedlings after 24 h. Growth and quantification were performed as described in (A). Five plates per genotypes with total > 100 seedlings were used for generating the graph (B). Values are means ± S.E. (C) Phototropic responses of hypocotyls of 4-days dark grown seedlings. Growth conditions and quantification were performed as described in (A). Four plates per genotype with total > 96 seedlings for Ws and > 75 seedlings for each *abp1* mutants we counted. Values are means ± S.E. Phototropism was induced by lateral blue light (10 µmol m^{-1} s^{-2}) for 8 hours from LED light (CFL, PlantClimatic, GmbH, http://www.plantclimatics.de). One arrow = *ABP1-OX*, two arrows = Ws.

These *abp1* mutants express no longer the *ABP1* wild type allele and were made homozygous for the mutant allele. We transformed also the 35S::*ABP1* wild type cDNA with tags constructed into heterozygous *abp1*/+, termed *ABP1-OX*. *abp1* mutants showed mutant properties when grown in white light and also in certain light conditions. *abp1* mutants as well as *ABP1-OX* showed a wavy pattern and slanting root growth

(Fig.1 A-B), and had longer hypocotyls (Fig.1 C) in comparison to wild-type. Hypocotyls and roots of *abp1* mutants were less responsive to gravity (Fig. 2 A). The hypocotyls of wild type seedlings showed a dominant single peak at 60° bending which was also observed for *ABP1-OX* plants whereas *abp1-8* and *abp1-9* produced a single peak at 50° and *abp1-10* had a peak at 40°. Similar results were obtained for gravitropic responses of roots where seedlings of *abp1-8*, *abp1-9* and *abp1-10* showed a weaker response by producing a peak in bending angles at 50° in *abp1-8* and *abp1-9*, and 40° in *abp1-10*. Wild type and *ABP1-OX* displayed a peak at 80° and 70° bending angle respectively (Fig. 2 B). We analyzed phototropism in *abp1* mutants by exposing 4 days old dark grown seedlings to lateral 10 µmol m^{-1} s^{-2} blue light for 8 h. We found hypocotyls of *abp1* mutants were less sensitive to blue light as compared to wild type and *ABP1-OX*, and bending angles were approximately 60° in *abp1* mutants and 70° in wild type and *ABP1-OX* (Fig. 2 C). From these data, it is obvious that all *abp1* mutants are less sensitive in gravitropism and phototropism in comparison to wild type whereas wild type cDNA overexpressing *ABP1-OX* showed no insensitivity.

***abp1* mutants exhibit insensitivity to auxin**

We tested sensitivity of *abp1* mutant to auxin by growing seedlings on agar media containing increasing auxin concentrations. Root length and lateral root number were analyzed. There were only small differences in main root length between wild type and *abp1* mutants observed at auxin concentrations of 0.01 µM – 0.05 µM, except *ABP1-OX* which showed slightly longer roots than wild-type at 0.01 µM – 0.1 µM (Fig. 3 A).

A significant decrease was found in lateral root numbers in all *abp1* mutants, particularly in response to auxin higher than 0.03 µM (Fig. 3 B). Wild type and *ABP1-OX* had more lateral roots in comparison to *abp1* mutants at these auxin concentrations. These data indicate lower auxin sensitivity in *abp1* mutants in comparison to wild type and *ABP1-OX* plants.

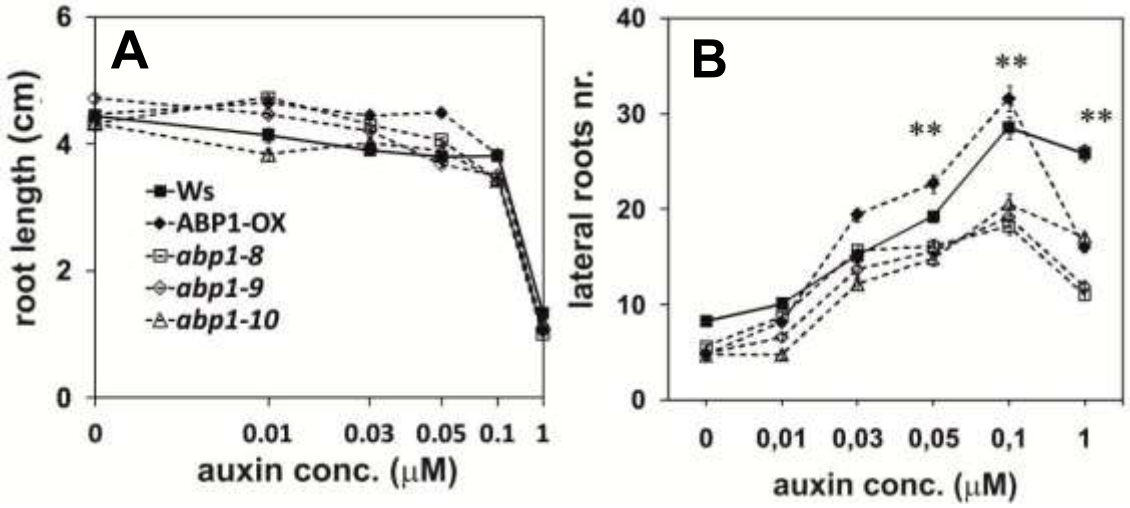

Figure 3. Auxin sensitivity of root response of Ws, *ABP1-OX* and *abp1* mutant seedlings.

Seedlings of Ws, *ABP1-OX* and *abp1* mutants were grown on vertical agar media without auxin for 4 days, then transferred to plates containing increasing auxin concentration for 6 more days before main root length was determined (A) and lateral root number (B). Data for each genotype were obtained from three plates with total n = 30. Experiments were repeated two times independently. Values are means with S.E. (differences were $p < 0.001$ for Ws and *ABP1-OX* versus *abp1* mutants indicating by **)

Lower auxin sensitivity is revealed in gene expression in the *abp1* mutants

Several previous studies have shown that most of auxin-related mutants confer severely defective phenotypes and are also impaired in auxin-induced gene expression (Park et al., 2002; Braun et al.,

2008; Effendi et al., 2011). Expression of seven early auxin-induced genes (*IAA2, IAA11, IAA14, IAA19, SAUR9, SAUR23, GH3.5,* and *ABP1*) and four PIN genes (*PIN1, PIN2, PIN3,* and *PIN5*) in *abp1* mutants, wild type and *ABP1-OX* was tested. We focused to measure expression level these genes at 0 min, 30 min and 1 hour after auxin treatment (Effendi et al., 2011).

At 30 minutes after treatment with 1 µM 1-NAA, seven (*IAA2, IAA11, IAA19, SAUR9, SAUR23, GH3.5,* and *ABP1*) of eight early auxin-responsive genes showed up-regulation in wild-type seedlings by approximately two to fivefold (Fig. 4). *abp1* mutants showed almost no up-regulation in most early auxin-responsive genes, a slight up-regulation less than twofold was found for *IAA2* and *IAA19* in all *abp1* mutants.

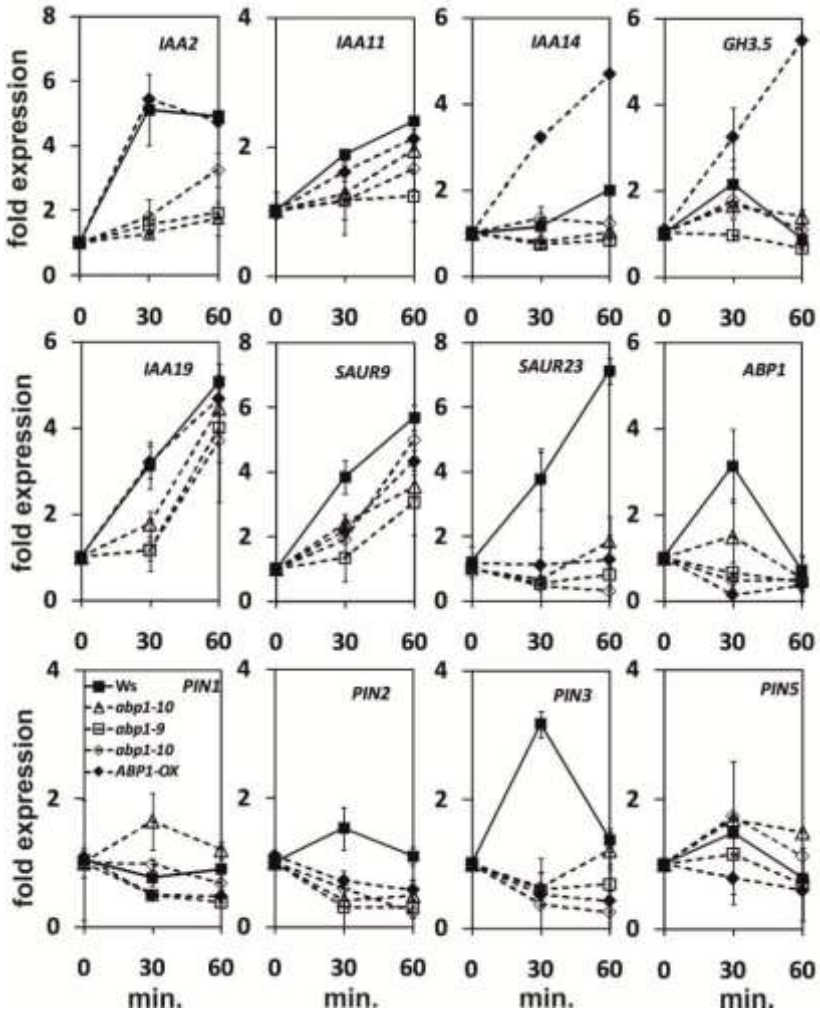

Figure 4. Transcriptional expression of early auxin genes and some PIN genes in light grown Ws, *ABP1-OX* and *abp1* mutants.

Seedlings were grown on ½ MS media agar on 8h/16h white condition for 14 days. Seedlings were then incubate on ½ MS liquid media for acclimatisation for 2 hours, then transferred and incubated in fresh ½ MS liquid media containing 1 µM 1-NAA for 0 min, 30 min and 60 min. Seedlings were dried as quickly as possible with tissue paper and frozen in liquid nitrogen. For detail of RNA extraction and cDNA synthesis, see Experimental Procedures. Quantitative Real Time PCR (qRT-PCR) data were obtained from three biological replications with three technical replications for each target gene. Statistical analysis was performed as described by Livak and Schmittgen (2001) and verified using the method as described by Pfaffl *et al.* (2002).

A slight up-regulation in *SAUR9* was observed in *abp1-10*, but lower in comparison to wild type. *ABP1-OX* showed up-regulation in five genes (*IAA2, IAA11, IAA14, IAA19,* and *GH3.5*) with similar fold

expression as in wild type. However, no up-regulation was found in *SAUR9, SAUR23* and *ABP1* in the *ABP1-OX*. We noticed that *abp1-8* and *abp1-9* as well as *ABP1-OX* seedlings showed down-regulation in *SAUR23* and *ABP1* genes after 30 minutes auxin treatment, while wild type showed up-regulation of these genes. In comparison to wild type, expression of *PIN2* and *PIN3* was down-regulated in *abp1* mutants and *ABP1-OX* seedlings, while in wild type these genes were up-regulated after 30 minutes auxin treatment. *PIN1* and *PIN5* expression was near identical in all genotypes and little or not at all influenced by auxin (Fig. 4).

Taken together, the lower transcription of early auxin-responsive genes and *PIN* genes in *abp1* mutants clearly indicate insensitivity to auxin in *abp1* mutants in comparison to wild type and in comparison to *ABP1-OX*.

Response to monochromatic continuous red and far-red lights in *abp1* mutants

Cross-talk between auxin and light in plant growth regulation has been intensively investigated, particularly, responses to shade light (Devlin et al., 2003; Vandenbusche et al., 2003; Roig-Villanova et al., 2007; Hortnischek et al., 2009; Sorin et al., 2009; Keuskamp et al., 2010). Since *abp1* mutant seedlings had longer hypocotyls under white light condition (Fig. 1 C) this suggested that *abp1* mutants could have defects in light responses. We investigated responses of *abp1* mutants to different monochromic light by growing seedlings for 1 day in the dark and for 3 days either in continuous 1 µmol m^{-1} s^{-2} red light or 1 µmol m^{-1} s^{-2} far-red light. Under red light, *abp1-8*, *abp1-9* and *abp1-10* seedlings showed significantly longer hypocotyls than wild type and *ABP1-OX* seedlings (Fig. 5 A,B) and the hypocotyl growth direction was more or less random in this light condition (Fig. 5 C). Interestingly, in *abp1-8* and *abp1-9*, the hypocotyls were even longer than in *phyB-9* mutant seedlings, while *abp1-10* showed a length similar to *phyB-9* mutant seedlings (Fig. 5 A,B).

Similar to the responses in red light, all *abp1* mutants seedlings displayed longer hypocotyls in continuous far red in comparison to wild type but shorter in comparison to *ABP1-OX* and *phyA-211* mutant seedlings (Fig. 5 D and E). Since hypocotyl elongation is inhibited by continuous far-red light in a fluence-dependent manner (Whitelam et al., 1993) except in *phyA* mutants, long hypocotyls in *abp1* mutants under far red light condition suggested that *abp1* mutants might convey defective PHYA-mediated responses. However, not all *phyA* deficiency responses in de-etiolated seedlings were observed in *abp1-8, abp1-9* and *abp1-10*. For examples, the *abp1* mutants and *ABP1-OX* displayed no apical hook and opened and expanded cotyledons like in wild type (Fig. 5 F). Moreover, red and far-red light are known to reduce gravitropism in hypocotyls leading to randomization of hypocotyl direction (Robson and Smith, 1996; Kim et al., 2011).

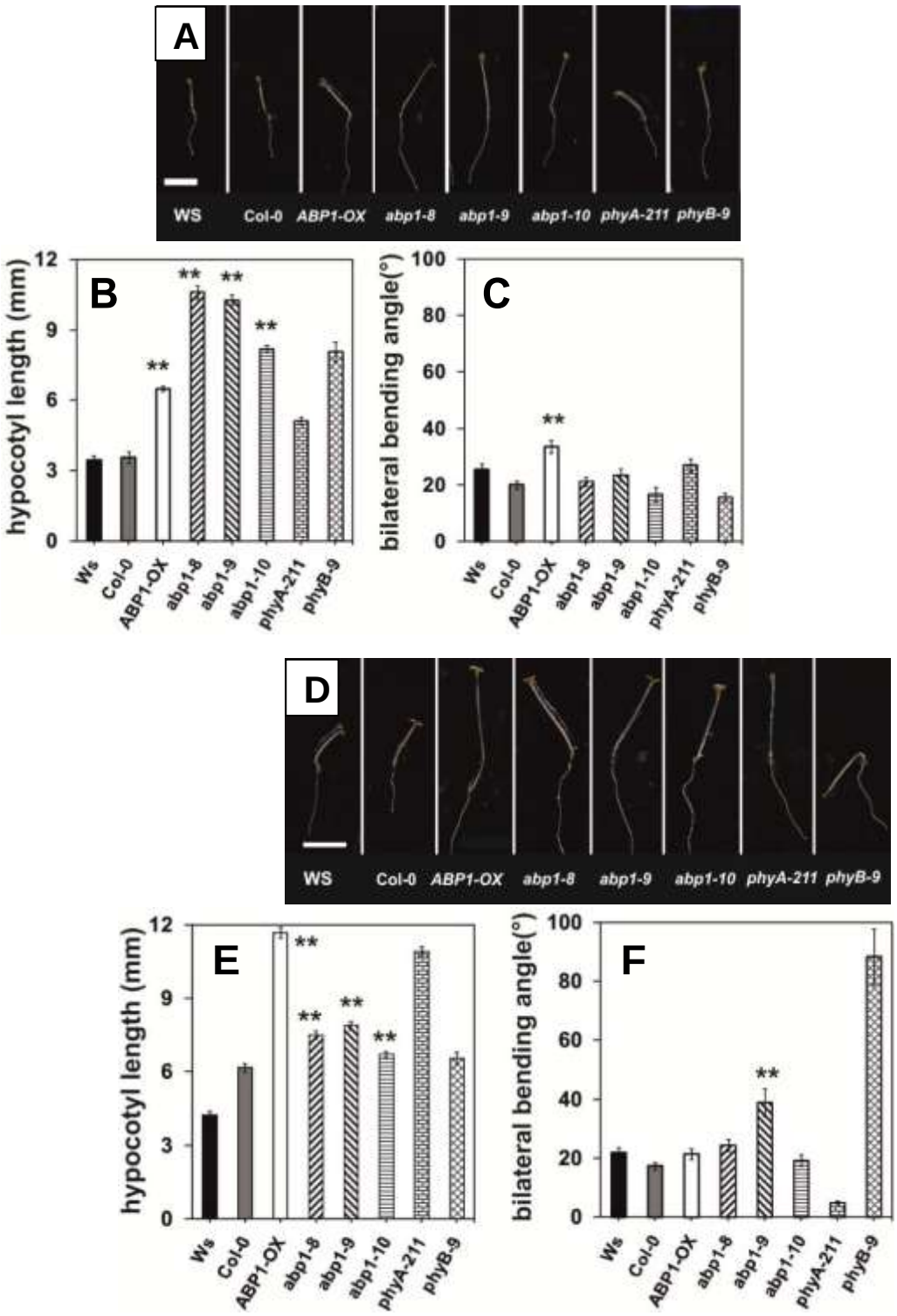

Figure 5. Responses of hypocotyl to monochromatic continuous red or far-red in Ws, *ABP1- OX*, *abp1* mutants, *phyA-211* and *phyB-9*. Representative images of red light-grown seedlings

(A) and far-red-grown seedlings (D). One day dark-grown seedlings were grown further under either with 1 µmol m^{-1} s^{-2} of red light or 1 µmol m^{-1} s^{-2} far-red light for 3 days on half strength MS Gelrite (Duchefa-Biochemie) media containing 1% sugar. Quantifications of hypocotyl length under red light and far-red light are shown in (B) and (E) respectively. The growth direction of hypocotyl under the same light condition was measured (red light, C) and (far- red, F). Experiments were repeated three times independently and each replication contains > 30 seedlings for each genotype. Values are means with ± S.E. ($p < 0.001$). Bar = 5 mm.

The data on growth direction of hypocotyls in *abp1* mutants again showed that not all *phyA* mutant properties are present in the *abp1* mutants. We found that hypocotyls of *abp1* mutants showed some randomization of growth direction in comparison to completely upright *phyA* mutant seedlings in far-red (Fig. 5 F) indicating only partialy insensitivity to far-red inhibition of gravitropism (Liscum and Hangarter, 1993; Robson and Smith, 1996) in *abp1* mutants. Thus, we suggest that *ABP1* is required only partially for far-red responses.

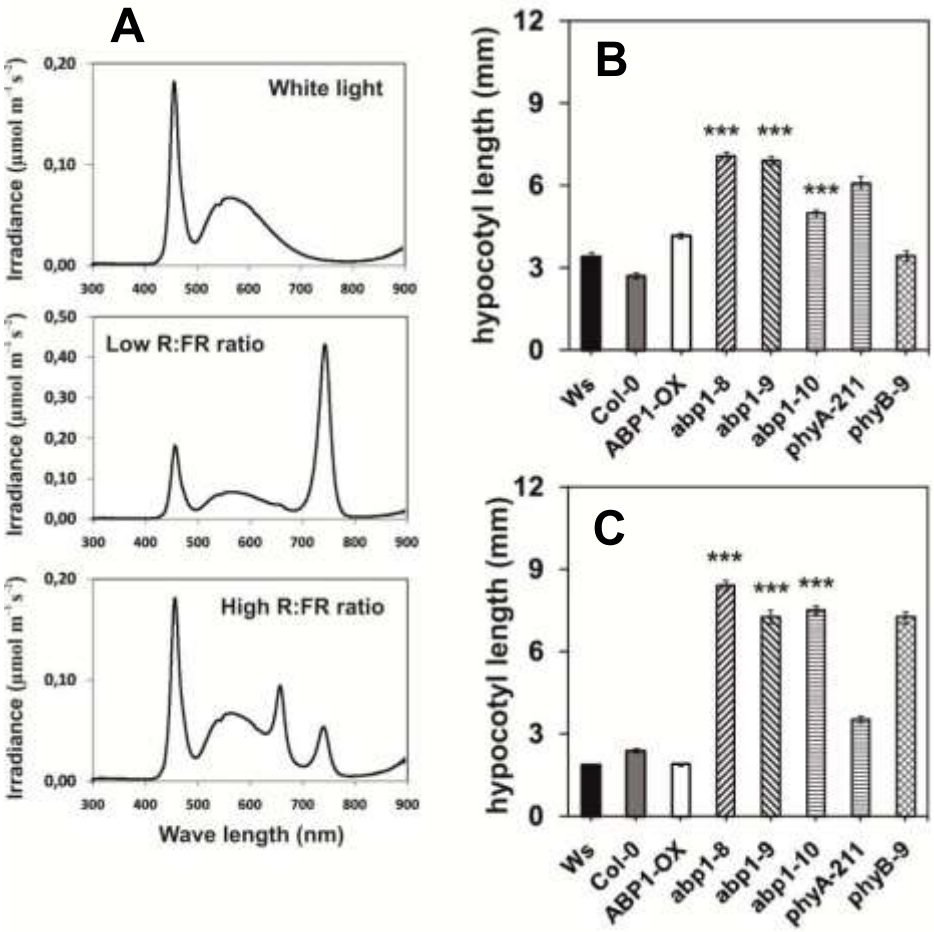

Figure 6. Responses of hypocotyl elongation in Ws, *ABP1-OX* and *abp1* mutants to far-red enriched light (low R:FR ratio) and red enriched light (high R:FR ratio).

(A) Light spectrum that were used in the experiments were measured using spectrometer USB4000 (Ocean Optic) and analyzed using software Spectrasuite (Ocean Optic). (B,C) Hypocotyl elongation in responses to low R:FR ratio or high R:FR ratio light. Seedlings were grown vertically on ½ MS agar media under constant white light (24.5 µmol m^{-1} s^{-2}) for 3 days, then added either with low R:FR ratio (0.098) (B) or with high R:FR ratio (2.1) (C) for 3 days more. Data were obtained from n > 120 seedlings for each genotype. Experiments were repeated independently 3 times with similar results and graphics were presented here represent one of three replications. Values are means with S.E. ($p < 0.001$).

***abp1* mutants are hypersensitive in response to shade-simulated light and flower early**

Changes in the ratio of red and far-red light are main cues for plants to pursue a strategy to avoid or tolerate this neighbor-induced light condition (Robson et al., 2010). The most dramatic response to shade light is hypocotyl elongation which can be remarkably rapid and start in only a few minutes (Ruberti et al., 2011). To investigate the response of *abp1* mutants to shade light, we grew *abp1* mutants seedlings under far red-rich light (low R:FR ratio) and red-rich light (high R:FR ratio) and analyzed the hypocotyl length. *Arabidopsis* seedlings were grown for 3 days under continuous white light (24,5 µmol m^{-1} s^{-2}) before a mixture of red and far-red light was added with either low R:FR ratio (0.098) or high R:FR ratio (2.1) for 3 more days (light spectrum in Fig. 6 A).

As shown in figure 6 B, *abp1* mutants seedlings displayed much longer hypocotyls under low R:FR ratio light, while wild type and *ABP1-OX* seedlings showed relative shorter hypocotyls. *abp1-8* and *abp1-9* mutants were even longer than the constitutive shade-avoidance *phyA-211* mutant seedlings. We also analyzed growth responses to high R:FR ratio in *abp1*mutants. Similarly insensitive responses as in low R:FR ratio data were observed in *abp1* mutants. They produced longer hypocotyls in comparison to wild type and *ABP1-OX* seedlings. Interestingly, *abp1* mutants showed a length similar to hypocotyls as displayed in the *phyB-9* mutant (Fig. 6 C). This indicated that *abp1* mutants might be defective in phyB-mediated responses to shade light, particularly in the hypocotyl length response. Shade responses are regulated redundantly by PHYB, PHYD, and PHYE (Franklin, 2008; Deng et al., 2010; reviewed in Stamm and Kumar, 2010).

Arabidopsis impaired in *phyB* function has a constitutively early flowering phenotype in short days (Halliday et al., 2003). We investigated flowering time in the *abp1* mutants in short days. All *abp1* mutants flowered 6-10 days ($p < 0.001$) earlier in comparison to wild type and two days earlier ($p < 0.05$) than *ABP1-OX* plants (Fig. 7 A and B). All *abp1* mutants produced a smaller rosette leaf number at flowering time compared to wild type and *ABP1-OX* (Fig. 7 C). Our flowering time data in *abp1* mutants support the notion that phytochrome-mediated mechanisms, particularly phyB-mediated signal mechanisms, are defect. We compared also leaf phenotypes of the *abp1* mutants to wild type as well as with *ABP1-OX*, since *phyB* mutation have been reported to have enhanced leaf area (Robson et al., 1993). We observed that all *abp1* mutants have longer and wider leaf blades in comparison to wild type and *ABP1-OX* (Fig. 7 D and F), suggesting *ABP1* might contribute to the repression of leaf blade expansion in the wild type and in the *abp1* mutants leaf expansion a phyB-regulated property might be also defect.

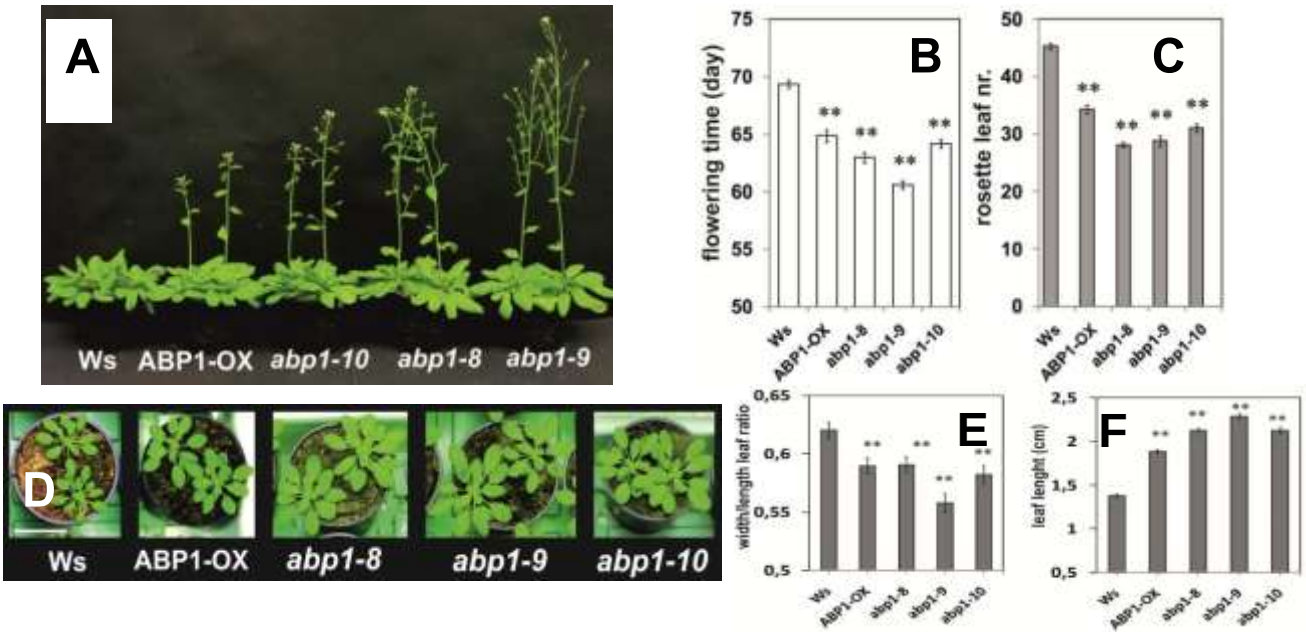

Figure 7. Early flowering phenotypes in in-vitro *abp1* mutants under short-days condition (8h/16h light/dark).

(A) Representative images of 59 days-old plants of Ws, *ABP1-OX*, *abp1* mutants (*abp1-10, abp1-8, abp1-9*). Plants were grown under short-days and flowering time was defined as the time of the first flower emerging which was indicated by opening of the first bud and white petals became visible. (B) Flowering date. (C) Rosette leaves number. Experiments were conducted in two independent replications. From each replication, 30 plants for each genotype were recorded for their flowering date and rosette leaf number. Values are means with S.E. (D). Representative images of leaves appearance of Ws, *ABP1-OX*, *abp1-8, abp1-9,* and *abp1-10.* Ratio of width:length of leaf blades (E) and length of leaf blade (F) were measured

from 59 days-old plants. Three biggest leaves from each plant were taken as samples and measured and data were obtained from 60 plants for each genotype. Values are means with S.E. ($p < 0.001$). Bar = 5 cm.

Taken together, the experiments in far red- and red-monochromatic light and shade light indicate defects in phyB-regulated responses but also in monochromatic light, a partial defect in phyA-regulated responses is indicated.

Transcriptional expression of light-induced genes in *abp1* mutants

abp1 mutants exhibited defects that were shown to be stronger in response to shade light. Thus, we reasoned that *ABP1* might also be required for transcriptional regulation of genes involved in shade responses. Several shade-responsive genes have been identified (Devlin et al., 2003; Salter et al., 2003; Sessa et al., 2005; Hortnischek et al., 2009) and some of them were known as primary targets in a shade-regulated transcriptional cascade (Carabelli et al., 1993, 1996; Steindler et al., 1999; Morelli and Ruberti, 2002; Roig-Villanova et al., 2006; review in Stamm and Kumar, 2010; review in Ruberti et al., 2011). To investigate whether mutated *ABP1* resulted in defective responses to shade light, we investigated transcriptional expression of nine shade-induced genes (*ATHB2, HFR1, PIL1, PIF1, PIF5, IAA19, IAA29, PIN3,* and *FIN219*). We used a modified shade light set-up condition as previously described in Wang *et al.* (2011) for our experiment to restrict light influence to a short induction period. Samples were prepared by growing seedlings on agar media under constant white light (24.5 µmol m^{-1} s^{-2}) for 7 days and then transferred to white light supplemented either with far-red (R:FR ratio of 0.098) for 1 hour (Fig. 8 A) or red (R:FR ratio 2.1) for 1 hour (Fig. 8 B).

Expression of tested far red light-induced genes in the *abp1* mutants was found to differ significantly from wild type in each mutant. 50%-90% of the tested genes were expressed statistically significant different. In response to FR-rich light (low R:FR ratio), a basically similar pattern in the expression of three shade-induced genes (*ATHB2, HFR1, PIL1*) was observed in wild type, *ABP1-OX* and in *abp1-8* (Fig.8 A). However, *abp1-9 and abp1-10* mutants expressed two or all three of these genes lower in comparison to wild type and *ABP1- OX*. *abp1-9* showed the lowest induction for both *ATHB2 and HFR1* genes, while in *abp1-10* only *HFR1* induction was low in comparison to wild type. No great differences were observed in the expression of *PIL1* between mutants and wild type, only *abp1-10* showed lower *PIL1* expression than wild type. Interestingly, *ABP1-OX* also showed low induction of *HFR1*, a gene which suppresses elongation to balance *ATHB2* (Sessa et al., 2005; Hortnischek et al., 2009). Higher induction was observed in *PIF1* expression in *abp1-10* in comparison to wild type, but the other *abp1* mutants were not significantly different in comparison to wild type.

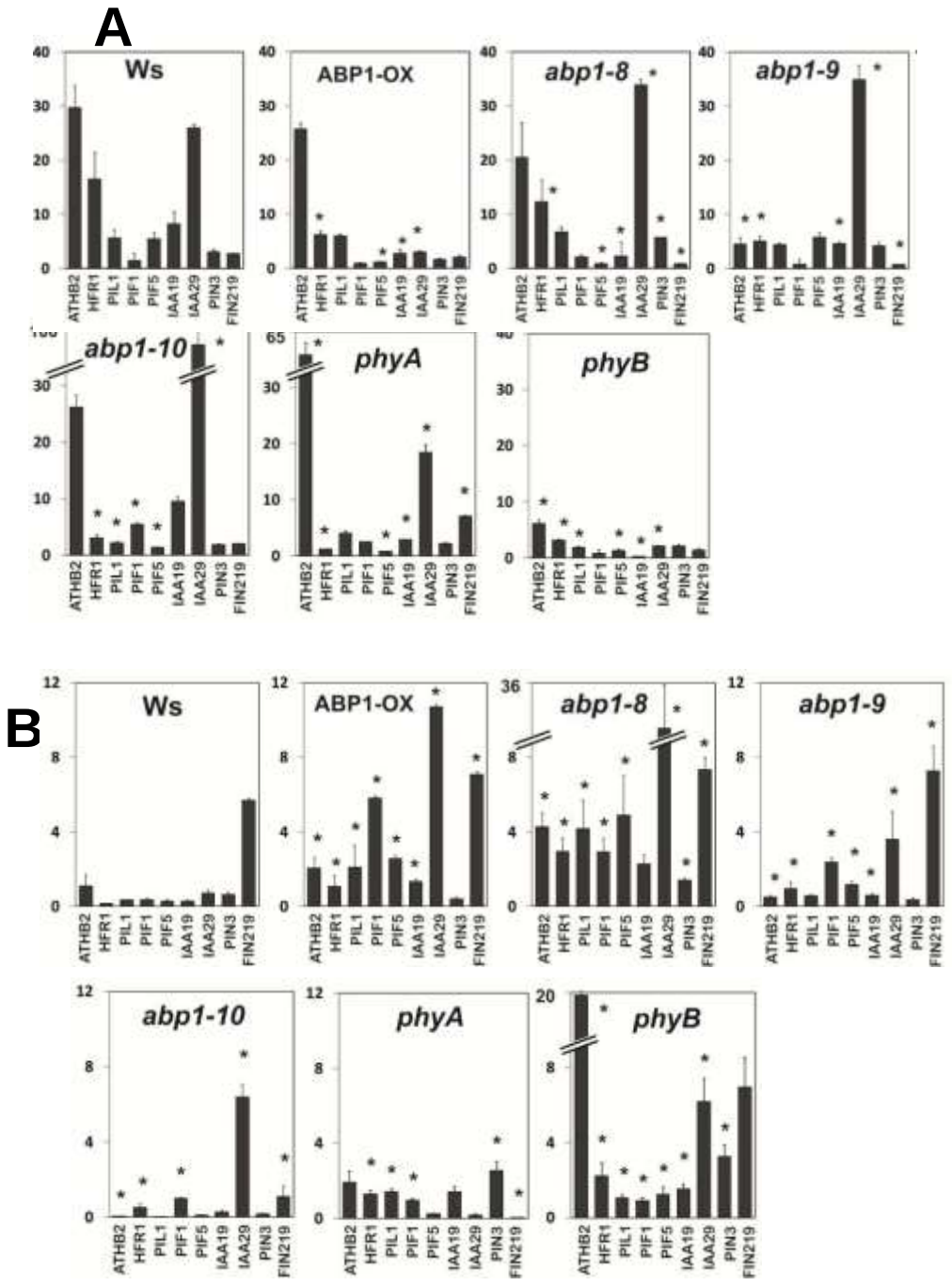

Figure 8. Transcriptional expression of light-induced genes under far-red riched- (A) and red riched-growth (B) condition in Ws, *ABP1-OX*, *abp1-8*, *abp1-9*, *abp1-10*, *phyA-211*, and *phyB-9* seedlings. Seedlings were grown 7 days under 24.5 m^{-1} s^{-2} white light before treatment with low R:FR ratio (0.098) or high R:FR ratio (2.1) for 1 hour. Seedlings were frozen and used for RNA extraction material. For detail of RNA extraction and cDNA synthesis, see Experimental Procedures. Quantitative Real Time PCR (qRT-PCR) data were obtained from at least three biological replications with three technical replications for each gene target. Statistical analysis was performed as described by Livak and Schmittgen (2001) and verified using method described by Pfaffl *et al*. (2002). Values are means with S.E. ($p < 0.05$). Significant differences (*) were relatively compared to Ws with at least $p < 0.05$.

PIF5 is positively up-regulated by low R:FR ratio light (Lorrain et al., 2008) as was observed in wild type and in *abp1-9* statistically significant, but not in other *abp1* mutants and *ABP1- OX*.

In *phyA-211*, *HFR1* induction was absent and *ATHB2* induction was very high. *PIF5*, *IAA19* and *IAA29* induction was low but *PIF1* was similar to wild type. Still with the exception of shade repressor *HFR1*, the expression pattern of *phyA-211* after far red induction was overall similar to wild type. In *phyB-9*, induction of all these genes was very low.

Expression of *IAA19* and *IAA29* genes was up-regulated by simulated shade light (Hortnischek et al., 2009). In our experiments, *IAA19* expression was variable in *abp1* mutants. *abp1-8* and *abp1-9* as well as *ABP1-OX* showed lower transcriptional levels of *IAA19* in comparison to wild type (Fig. 8 A), while *abp1-10* showed induction similar to wild type. Moreover, we found that *IAA29* expression was similar in all *abp1* mutants, *phyA-211* and wild type; only in *ABP1-OX* induction was lower just as in *phyB* (Fig. 8 A). We found slight up-regulation in *PIN3* expression in all genotypes, but only *abp1-8* and *abp1-9* showed a slightly higher induction of *PIN3* than wild type (Fig. 8 A). All together, it is obvious that *abp1* mutants and, to some extent *ABP1-OX*, have quantitative reductions in expression of shade-induced genes compared to wild type, suggesting that *ABP1* is involved in the mediating of shade avoidance responses which is controlled mainly by *phyB*.

Under red-rich light (high R:FR ratio), all tested genes, except *FIN219*, were not up-regulated in wild type, while *abp1* mutants displayed various expression patterns under the same light condition (Fig. 8 B). *ABP1-OX* and *abp1-8* showed induction in almost all genes by red light addition (Fig. 8 B), while *abp1-9* and *abp1-10* displayed only slight elevation in transcriptional levels of few genes or were similar to wild type. Similar gene expression patterns were observed in *ABP1-OX* and *abp1-8*, and the generally high induction by added red light of many of the tested genes was also found in *phyB-9*. In high red, lack of suppression of the tested genes is apparent in *phyB-9*. As general pattern in *phyB-9* and *ABP1- OX* and *abp1* mutants, relatively high induction of *IAA29*, low induction of *PIN3*, and high induction of *FIN219* was found. Only *FIN219* was induced in wild type so that induction of *IAA29* and *PIN3* may be a "signature" for *phyB-9* which was also found in *abp1* mutants.

DISCUSSION

Choosing engineered point mutations is convenient to investigate ABP1 functions. With heterozygous *abp1/+* we could perform our experiments only with seed mixtures of 2:1 heterozygous:wild-type (Effendi et al., 2011). Because the loss of function of *ABP1* in homozygous plants is embryo lethal (Chen et al., 2001) viable homozygous *abp1* mutants should provide a better chance in experimental handling and in obtaining new phenotypes. Here we present three viable, engineered *abp1* mutant lines, *abp1-8*, *abp1-9*, and *abp1-10*, containing mutations in the auxin binding domain (Woo et al., 2002; Napier 2002) and expressing no wild-type *ABP1* allele in *Arabidopsis*. Choosing to mutate the residues Thr54 to Ile54 in *abp1-8* and Leu25 to Tyr25 in *abp1-9* in the binding domain for auxin (Woo et al., 2002) might change binding of auxin to ABP1, and mutation in His106 to Asn106 in *abp1-10* might change binding to the zinc ion in ABP1 and indirectly of auxin to zinc, similarly as in *abp1-5* where another zinc chelator, His59, is mutated (Robert et al., 2010). Those other artificial mutations were tried by us which involved the Trp151 residue proved non-viable plants. This tryptophan was shown to be highly important for function (David et al., 2007). As a receptor, proteins should bind ligands with strict structural and steric specificity. Mutations of critical amino acids in the presumed receptor will affect signal transduction and the downstream functions (Jones and Sussman, 2009) as shown here.

abp1 mutants exhibit altered developmental responses to auxin which resemble a hyposensitive phenotype

Our vector was constructed to code for an inserted strep-flag double tag right before the ER retention signal KDEL so that even expression of the wild type cDNA in the construct in *ABP1-OX* potentially could confer mutant properties. Expression of all mutated *abp1* cDNAs in *ABP1*-null background indeed showed impaired responses in auxin-related functions but not expression of wild type cDNA in *ABP1-OX*. From plants expressing additional point mutations we expected stronger auxin-related phenotypes. All mutants shown here had strong slanting root angles and waving roots (Fig. 1). Plants with strong slanting and waving root phenotypes often have reduced gravitropic responses (Okada and Shimura, 1990; Luschnig et al., 1998) as well as other auxin-related function such as a reduction in auxin sensitivity (Simmons et al., 1995; Sedbrook et al., 1999; Ferrari et al., 2000; Sedbrook et al., 2002; Santner and Watson, 2006; Sedbrook and Kaloriti, 2008; Effendi et al., 2011). Additionally, under our experiment conditions, *abp1* mutants had longer hypocotyls than wild type indicating defects in auxin functions or in light signaling or both (Liscum and Hangarter, 1991; review in Halliday et al., 2009). Light-related functions of ABP1 will be discussed below.

Hyposensitivity to exogenous auxin was tested in the classical root responses; main root growth inhibition and lateral root formation and in testing rapid induction of auxin-regulated genes. Whereas *ABP1-OX* was like wild type in developmental responses all point mutants were hyposensitive to auxin (Fig. 2). Using gene regulation as a test, delayed regulation was evident in the point mutations but also in a few genes in *ABP1-OX* (Fig. 3). Exogenous auxin did not evoke altered developmental responses in *abp1-5* or *abp1-SS12K* (Braun et al., 2008; Robert et al., 2010) or *abp1/+* (Effendi et al., 2011) but delayed gene regulation was also found in them (Effendi et al., 2011; Effendi et al., 2012-submitted). Defects in early auxin- induced gene regulation had proven to be a sensitive tool to identify functional defects in *abp1* mutants before (Effendi et al., 2011; Effendi and Scherer, 2011; Effendi et al., 2012- submitted). So, the data were presented here described new mutants have stronger auxin- related phenotypes than previous ones manifested in morphological responses and regulatory responses.

abp1 mutants are defect in phototropism and gravitropism

Regulation of auxin transport from cell to cell via polar auxin transport mechanism is suggested to start tropic responses (Friml et al., 2002; Esmon et al., 2005; Esmon et al., 2006; Petrásek et al., 2006; Rakusova et al., 2011). PIN2 and PIN3 were identified mainly mediating tropic responses (Müller et al., 1998; Friml et al., 2002). Recent experimental evidence (Wisniewska et al., 2006; Abas et al., 2006; Robert et al., 2010; Xu et al., 2010; Deng et al., 2011; Effendi et al., 2011; Effendi and Scherer, 2011; Effendi et al., 2012-submitted) and our data presented in this study indicate that ABP1 could mediate regulation of auxin transport in tropism by rapid changes in PIN subcellular distribution. We therefore suggest that ABP1 acts through the activity changes of PIN proteins induced by endocytosis and transcytosis (Klein- Vehn and Friml, 2008).

Transcriptional regulation could become important for a more sustained type of response. In line with this are low transcript levels of *PIN2* and *PIN3* in responses to auxin in the *abp1* mutants so that a decreased phototropic and gravitropic response of roots and hypocotyls in the *abp1* mutants can not be sustained (Fig. 3). Among genes that were identified as *Tropic*

Stimulus-Induced (TSI) are auxin-dependent genes activated in *Brassica oleracea* (Esmon et al., 2006). Among these are *GH3.5* and *IAA19* which are expressed lower in *abp1* mutants in response to auxin (Fig. 3). Repressor protein IAA19 has been identified to be involved in the regulatory feedback loop for the control of auxin-dependent tropic responses by making heterodimer with ARF7. Lacking activity of auxin-regulated transcriptional activator NPH4/ARF7 in *Arabidopsis* seedling will promote the disruption of photo- and gravitropic responses (Liscum and Briggs, 1996; Watahiki and Yamamoto, 1997; Stowas-evans et al., 1998; Watahiki et al., 1999). Although current knowledge of transcriptional regulation of auxin-responsive genes identify the receptor TIR1 as the key player in gene regulation (Mockaitis and Estelle, 2008), recent studies have demonstrated that ABP1 may contribute in the regulation of early auxin-responsive genes (Braun et al., 2008; Tromas et al., 2009; Effendi et al., 2011; Effendi et al., 2012-submitted). Taken together, we suggest that

ABP1 contributes to the control of gravitropic and phototropic responses by modulating PIN action and regulating the expression of some auxin-induced Tropic Stimulated-Induced genes.

abp1 mutants are red-insensitive in response to monochromatic light

The *abp1* mutants were investigated here, having a stronger auxin-related phenotype as those were investigated before (Braun et al., 2008; Robert et al., 2010; Xu et al., 2010; Effendi et al., 2011; Effendi et al., 2012-submitted). Surprisingly, the *abp1* mutants were all insensitive to monochromatic red and far red light (Fig. 6). There seems to be only few mutants *like pft1 (phytochrome and flowering time1)*, *prr7 (pseudo-response regulator7)* and *rf2-1* (*red and far-red insensitive2 to 1*), which is insensitive to both light condition (Cerdán and Chory, 2003; Kaczorowski et al., 2003; Chen and Ni, 2006). As in all other red or far red light experiments, *ABP1-OX* had a mutant phenotype as well, not only the *abp1* mutants, which was usually clearly weaker than in *abp1* mutants. We assume that the tags which were inserted closely to the mobile C-terminus of ABP1 confers conformational change(s) which cause slightly aberrant signal transduction (Bertośa et al., 2008; Scherer, 2011), especially, in pathways leading to interaction with red light signaling. The auxin-induced responses seemed to be less affected or not at all by this presence of a tag.

***abp1* mutants show constitutive or hypersensitive response to shade light conditions**

Surprisingly, *abp1* mutants showed constitutive shade responses. As "shade" is defined white light enriched with far red light i.e. having a low ratio of R:FR. Using phytochromes, plants are able to detect the presence of neighboring plants by monitoring the change in R:FR ratio of light (Franklin, 2008). Reduction in the R:FR ratio due to selective absorption of red light by photosynthetic pigments (Ballaré et al., 1990) is happened in shade from neighboring plants. Thus, the plants respond to this condition by promoting a complex growth mechanism for obtaining more light which, in summary, are known as shade avoidance responses, such as hypocotyl and shoot elongation, petiole elongation, leaf hyponasty and early flowering (Franklin, 2008; Lau and Deng, 2010; Stamm and Kumar, 2010).

The long hypocotyls of *abp1* mutants grown under monochromatic red or far red light respectively (Fig. 4) indicate that *PHYB*- and *PHYA*-related functions, respectively, could be impair in the *abp1* mutants. Plants with similar phenotypes are often having defectives in *phyB* or *phyA* respectively (McCormac et al., 1993; Whitelam et al., 1993). In fact, *abp1* mutants show longer hypocotyl than single *phyB-9* or *phyA-211* mutant, suggesting that both *phyA*- and *phyB*-related functions could be defect. In comparison to single mutant *phyA* or *phyB*, *phyAphyB* double mutants grown under any monochromatic and mixture of R:FR light radiation have taller hypocotyls (Smith and Whitelam, 1997). In line with this notion, the early flowering time and wider and longer leaf blades in the *abp1* mutants (Fig. 5 and 6) provide evidences that particularly *PHYB*-related functions are defect (Halliday et al., 2003; Robson et al., 1993; Keller et al., 2011) since low signaling activity of PHYB is the basis for the shade avoidance responses (Casal, 2012).

***abp1* mutants misregulate far red and red light-induced genes**

abp1 mutants are red light-signaling mutants and misregulated about two-third of the shade- induced genes we tested here (Fig. 8). How exactly auxin is involved in light signaling, particularly in shade responses, remains as yet unknown (Franklin, 2008; Stamm and Kumar, 2010; Keller et al., 2011; Nozue et al., 2011). One suggested mechanism is induction of auxin biosynthesis by TAA1 in shade (Tao et al., 2008). Other mechanisms are gene regulation (review in Halliday et al., 2009; review in Ruberti et al, 2011) and diversion of polar auxin transport to regulate growth. Obviously, all three mechanisms could or even should be cooperating (Steindler et al., 1999; Hsieh et al., 2000; Devlin et al., 2003; Salter et al, 2003; Sessa et al., 2005; Carabelli et al., 2006; Lorrain et al, 2007; Roig-Villanova et al., 2007; Hortnischek et al., 2009; Keuskamp et al., 2010).

We used shade-induced genes as indicator genes and as a means to identify defects in light- induced gene regulation in the *abp1* mutants. *ATHB2, HFR1, PIL1, PIF1, PIF5, IAA19, IAA29, PIN3,* and *FIN219* are far red- or shade-dependent genes (Steindler et al., 1999; Devlin et al., 2003; Salter et al, 2003; Sessa et al., 2005; Lorrain et al, 2007; Roig-Villanova et al., 2007; Hortnischek et al., 2009; Keuskamp et al., 2010). *ATHB2, IAA19, IAA29, PIN3* and *FIN219* were also identified as auxin-related genes (Steindler et al., 1999; Devlin et al., 2003; Roig-Villanova et al., 2007; Hortnischek et al., 2009; Keuskamp et al., 2010). In *phyA-211* plants, tall hypocotyls in this light are correlated with high transcript levels of *ATHB2, HFR1,* and *IAA29* (Fig. 7). Of these genes, high level of *ATHB2* transcript could be a cue for hypocotyl elongation under shade light (Schena et al., 1993; Steindler et al., 1999; Carabelli et al., 2006) and it is known as a positive regulator for hypocotyl elongation (Kunihiro et al., 2011). However, as a negative regulator of shade responses, *HFR1* inhibits the action of *ATHB2* (Sessa et al., 2005; Hortnischek et al., 2009), to prevent exaggerated hypocotyl elongation under shade light. The low transcription level of *HFR1* in the *phyA-211* and in the *abp1* mutants might provide the even more importance cue in determining hypocotyl elongation rather than a high level of *ATHB2* alone. Exception is *abp1-8* were both *ATHB2* and *HFR1* genes were at low level induced. ATHB2/HFR1 interaction is part of the gas and brake mechanism of positive and negative regulators of shade avoidance responses (Sessa et al., 2005; Jiao et al., 2007) which in the *abp1* mutants could be defect at least at the level of *ATHB2* and *HFR1* expression.

After short induction by red light added to white light a "signature" of a PHYB-like response could be the high-low-high expression pattern of *IAA29-PIN3-FIN219* (Fig. 8) in *phyB-9* which, to some extent similarly, was found in the *abp1* mutants including the *ABP1-OX*. *phyB-9* and the *abp1* mutants and *ABP1-OX* all exhibited tall hypocotyls in red light while wild type and *phyA-211* did not showed it and had low *IAA29* expression. These features indicate that red light-dependent light genes are regulated similarly in the *abp1* mutants and in *phyB-9*. Moreover, this high-low-high pattern was also found in the far red light condition where *phyA-211* grew tall hypocotyls. Noteworthy, *IAA29* has been shown to be a component of auxin-mediated elongation growth in shade avoidance responses (Tao et al., 2008). The *taa1/wei1/sav3* mutant has a defect in the locus encoding the TAA1 protein involved in IAA biosynthesis. This mutant also shows a reduction in transcript level of *IAA29* and is unable to elongate in simulated shade light (Tao et al., 2008). Thus, high level of *IAA29* transcript in *abp1* mutants as well as in the *phyB-9* response to red light but not in wild type correlated with tall hypocotyl. High expression of *FIN219* correlated with a tall hypocotyl (Wang et al., 2011), here seen correlated in *phyA-211* in far red, and red in *phyB-9* and the *abp1* lines. Contradicting this correlation is the high expression in wild type in red but there the low *IAA29* expression may not allow a long hypocotyl. This complicated regulatory interaction of red light-activated genes (Jiao et al., 2007) may not allow a simple straightforward explanation of the transcription results but, clearly, the *abp1* mutants had aberrant red- and far red-induced gene expression which provides an explanation of the observed response phenotypes.

Shade avoidance encompasses also leaf expansion, petiole length and early flowering all of which are regulated by low signaling activity of PHYB redundantly together with PHYD and PHYE in comparable manner (Smith and Whitelam, 1997; Devlin et al., 1998; 1999; Franklin et al., 2003; Hornitschek et al., 2009). This corresponds to phenotypes exhibited in leaf expansion and early flowering (Fig. 7 B) in the *abp1* mutants and the weak phenotype of *ABP1-OX* (Fig. 7 D-F).

Recently, a new model of interaction between light and auxin has been put forward (Keuskamp et al., 2010; 2011) based on previous postulates of a diversion of the polar auxin transport by shade from inner tissue to the epidermis (Morelli and Ruberti, 2000, 2002; Ruberti, 2002). Redistribution of PIN3 subcellular distribution from basal to lateral plasma membranes of endodermal cells is hypothesized to induce lateral auxin transport from the inner cells toward the more lateral cell layers which, in turn, is supposed to lead to hypocotyl elongation. *PIN3* expression is enhanced by shade light in the course of several days (Keuskamp et al., 2010). Our short duration far red or red light did not strongly induce *PIN3* transcription but interaction of ABP1 with auxin polar transport has been proposed (Robert et al., 2010; Xu et al., 2010; Effendi et al., 2011) and the

regulation of transcriptional expression of *PIN* genes via ABP1 action were also addressed (data in this paper, Effendi and Scherer, 2011; Effendi et al., 2012-submitted).

Our postulated model for the ABP1 main function is that ABP1 is involved in regulating PIN protein activity, likely by protein phosphorylation and other cytosolic reactions, including phospholipase A activation (Effendi et al., 2011; Scherer et al., 2012). Likely, other auxin transport proteins could also regulated by ABP1 but experimental evidence for this is lacking. We assume that regulation of auxin transport regulates auxin concentration so that TIR1 regulates auxin-induced genes correspondingly. Hence, consistent with this hypothesis, ABP1 also controls other functions which need regulation of polar auxin transport, like phototropism and gravitropism (Effendi et al., 2011; data this paper). If diversion and regulation of polar auxin transport proves to be a main component of the shade avoidance responses this would explain why not TIR1 (Effendi et al., 2012-submitted) but ABP1 is the auxin receptor involved in shade avoidance.

EXPERIMENTAL PROCEDURES

Plant material and growth condition

Arabidopsis thaliana Wassilevskija (Ws) and Columbia (Col-0) wild type plants were used for transformation. Light mutants *phyA-211* (Col) was obtained from C. Luschnig (BOKU, Vienna - Sweden). *ABP1* cDNA containing flag-tag and strep-tag II directly prior to the C- terminal KDEL under control of the 35S promoter was kindly provided by T. Reinard – Institute of Plant Genetics, University of Hannover. This construct was then cloned into pENTR D-TOPO (Invitrogen) where site-directed mutation was performed using QuikChange™ Site-Directed Mutagenesis Kit (Stratagen). Entry vectors were cloned into destination vector pB2GW7 (Karimi et al., 2002) and transformed into *Arabidopsis thaliana* heterozygous *abp1/+* (Chen et al., 2001). Progenies of the transformed plants were selected on agar plates containing kanamycin (50µg/ml) and BASTA (30µg/ml). Surviving seedlings were grown further on soil and PCR genotyping to identify homozygous null ABP1 wild type plants. PCR genotyping was done using reverse ABP1 genomic primer (5'-CCT GAG ATC TCA AGT AGG AAG CGT C-3') and right border primer (5‟-TCC CAA CAG TTG CGC ACC TGA ATG-3‟) primer (Chen et al., 2001).

Most experiments were performed on sterile agar or liquid half-strength Murashige and Skoog (MS) media. Seeds were surface sterilized, stratified for 4 days at 4°C, and germinated on 10cm X 10cm square plates containing half-strength basal salt mixture supplemented with 1% sucrose and either 1% agar or 0.5% Gelrite (Duchofa-Biochemie). Seedlings grown on plates then were used for various treatments. Phototropism was performed by placing the plates in darkness for 4 days before applying with 10 µmol m^{-1} s^{-2} lateral blue light for 8 hours (LED chamber, PlantClimatics). For gravitropism experiment, plates were placed for 4 days in the dark, then were tilted by 90° for 24 h. Experiments were repeated three times independently and each replication consisted of more than 90 seedlings for Ws and 75 seedlings for each of the *abp1* mutants. Quantifications were done by scanning the plates with CanonScan 8800F (resolution of 600 dots per inch; Canon, http://www.canon-europe.com) and evaluating lengths or angles with AXIOVISIO LE version 4.6 software (Zeiss, http://www.zeiss.com/).

Auxin sensitivity

Four days old seedlings were transferred to fresh half-strength MS agar media containing increasing 1-NAA concentrations, 0.01 µM, 0.03 µM, 0.05 µM, 0.1 µM, 0.3 µM and 1.0 µM without 1-NAA. For each concentration, thirty seedlings were used and the experiment was repeated two times. The plates were placed in a vertical position at 22°C under constant white light (50 m^{-1} s^{-2}) and 16/8h (light/dark condition) for 6 days, then scanned and quantified as described in the *plant material and growth condition* section. Data were analyzed using the t- test in Microsoft Excel.

Light condition and shade avoidance experiments

Seeds were prepared as described in plant material and growth condition above. After 4 days stratification, plates were placed in a horizontal position at 22°C under white light for 2 h before transfer for 1 day into darkness. Then they were treated either with 1 µmol m^{-1} s^{-2} red or 1 µmol m^{-1} s^{-2} far red for 3 days. All these light condition were prepared in a LED light chamber (CLF, PlantClimatics). For shade avoidance experiment, the plates were prepared like above and placed in the LED light chamber and exposed to 24.5 µmol m^{-1} s^{-2} constant white LED light for 3 days. Following this treatment either low R/FR ratio (0.098) or high R/FR ratio (2.1) was added for 3 days. Light spectrum was measured using spectrometer USB4000 (Ocean Optic) and analyzed using software Spectrasuite (Ocean Optic). For monochromatic light treatments, hypocotyls lengths and hypocotyls bilateral dropping angles were measured while for shade avoidance experiment hypocotyl length was quantified. Quantification was performed as described in the previous section in *plant material and growth condition* above. Data were obtained from three independent replications and each replication was consisted of more than 40 seedlings. Culture in soil was performed in the greenhouse. For flowering time data collection and *Arabidopsis* was maintained at 22°C constant, 16h/8h (light/dark condition) on peat-based compost soil (Einheitserde, http://www. einheitserde.de/) containing 30% silica sand. Flowering time was defined as the time of the first flower arising which was indicated by opening of the first bud and white petal are shown (Effendi et al., 2011). Ratio of width:length of the leaf was measured from adult plants.

Nucleic acid analysis

For transcriptional expression measurements seedlings were grown in half-strength MS agar medium for 14 days at 22°C under long-day conditions (16h/8h, light/dark condition). Then the seedlings were carefully transferred into fresh half-strength liquid medium for equilibration with gently shaking for 1 h. The medium was replaced by fresh liquid half- strength MS medium with 1µM 1-NAA for 60 min. Seedlings were blotted on filter paper and frozen in liquid nitrogen for further use. For transcriptional expression measurement of red light responses, after 4 days stratification at 4°C, the seedlings were grown at 22°C in a LED light chamber (CLF, PlantClimatics) 7 days under 24.5 m^{-1} s^{-2} continuous white light. Then R/FR mix light was added either with low R/FR ratio (0.098) or high R/FR ratio (2.1) for 1 hour (light spectra in Fig. 6 A). Samples were blotted and frozen in liquid and used for RNA extraction. For quantitative RT-PCR, 4-5 µg of total RNA was prepared using a TRIzol modified method (Maniatis et al., 1989) and transcribed to first-strand cDNA using a RevertAidTM H Minus first-strand cDNA synthesis kit (Fermentas, http://www.fermentas. com). Primers were designed and selected using PRIMER 3 software (http://frodo.wi.mit. edu/) and checked against primer dimer formation and primer efficiency using NETPRIMER software (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch.html). Trans- criptional expression measurements and the primers of qRT-PCR for auxin treatments were described in Effendi *et al.* (2011). The primers for shade avoidance were described in supplementary table 1. PCR efficiency of the primers was > 99%. Data were collected from two to three biological repeats and three technical replicates for each determination. Relative expression was calculated according to the ΔΔCt method (Livak and Schmittgen, 2001) and relative to 18S rRNA expression. The expression level for the control treatment was set as 1- fold. REST 2008 software (Pfaffl et al., 2002) was used for verify the statistical analysis. Means are statistically significantly different when error bars do not overlap ($p < 0.05$ or lower).

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