Isolation and Characterization of New Point Mutants of AUXIN BINDING PROTEIN 1

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Abstract

We show that the heterozygous *abp1/ABP1* has defect in auxin physiology-related responses and lower transcript level of early auxin-regulated genes (Effendi, *et al.*, 2011. Plant J. 65:285). We designed two mutants, M7 and M8, by introducing a mutated ABP1 cDNA, coupled to 35S promoter, into heterozygous *abp1/ABP1* plants and screened for null WT gene transcription in the progeny. We also isolated transgenic plant expressing WT ABP1 cDNA coupled to 35S (ABP1-OEX). M7 and M8 produced slightly shoter main root but fewer lateral root, in response to auxin. They showed slowed hypocotyl phototropism and slowed root and hypocotyl gravitropism, which ABP1-OEX did not show. M7 and M8 flowered early in SD but not ABP1-OEX. We also investigated the *abp1-5* (H94>T94) point mutant. *abp1-5* showed slowed root gravitropism but hypocotyl phototropism or gravitropism was not changed and it flowered at the same time as Col-0 in SD. *ABP1* point mutants will be a valuable tool in auxin research.

Point mutants M7 and M8 showed defects in response to auxin and less sensitive in regulation of early auxin-regulated genes

Early flowered in M7 and M8

Fig. 1. Phenotypes of the 43 days old LD-grown plants (A) Mutants M7 and M8 flowered earlier in comparison to OEX-ABP1 and wt. Statistic difference of flowering time is showed in the fig (B). M7 and M8 produced fewer rosette (fig.C) but have more shoots in comparison to wt (fig.1D) which indicate a defect in apical dominance. Asterix indicates significantly difference ($p < 0.001$, in A and B; $p < 0.05$ in D) relative to OEX-ABP1.

Lower number of lateral root in response to different auxin concentration in mutant M7 and M8

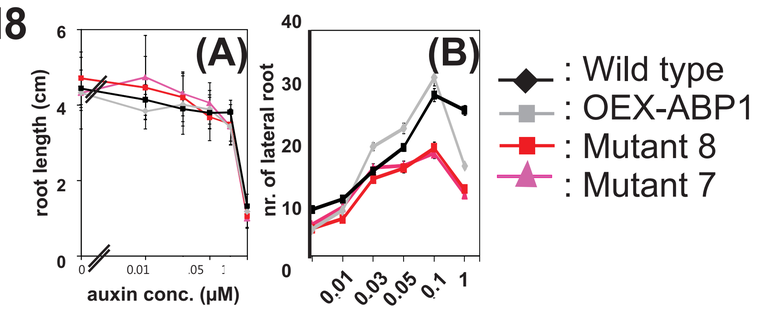


Fig. 2. Auxin sensitivity. (A) No difference in hypocotyls length was observed between M7, M8 to OEX-ABP1 or WT in response to different auxin concentrations (0.001µM, 0.01µM, 0.02µM, 0.03µM, 0,1µM,0.3µM, and 1µM) (B). However, M7 and M8 produced lower number of lateral root in comparison to OEX-ABP1 and wt. Plants were grown on agar plates for 4 days under normal light before were transferd to auxin-contained agar for 3 days. (for each auxin concentration n: 30)

Less response to gravitropism and phototropism in mutants M7 and M8

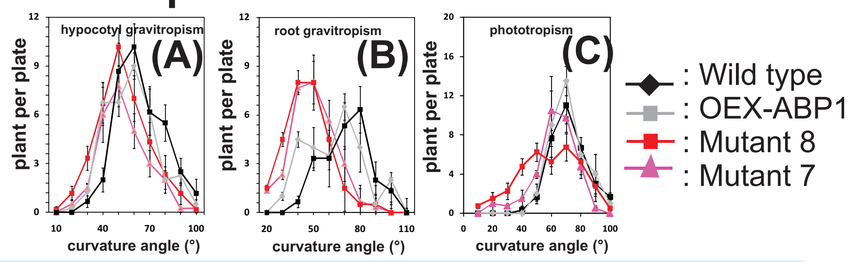


Fig.3. Response of hypocotyls and/or roots to gravity and light of mutants M7 and M8 seedlings. (A) The gravitropic response of hypocotyls of M7 and M8 showed lower responses to gravity by producing bending angles peak at 50°, whereas OEX-ABP1 and wt are 60°.(B) Similar response was observed on root gravitropism where M7 and M8 respond smaller to gravity in comparison to OEX-ABP1 and wt. 4 day dark-grown seedlings were grown on agar plates and were tilted 90° 24 h. (C) M7 and M8 produced smaller bending angles in response to 8 h 10µmol $m^{-2}s^{-1}$ lateral blue light in comparison to OEX-ABP1 and wt. Plants were grown 3 days on dark before applied with 10µmol $m^{-2}s^{-1}$ of lateral blue light.

Lower transcript abundance of early auxin-regulated genes and PIN genes in M7 and M8

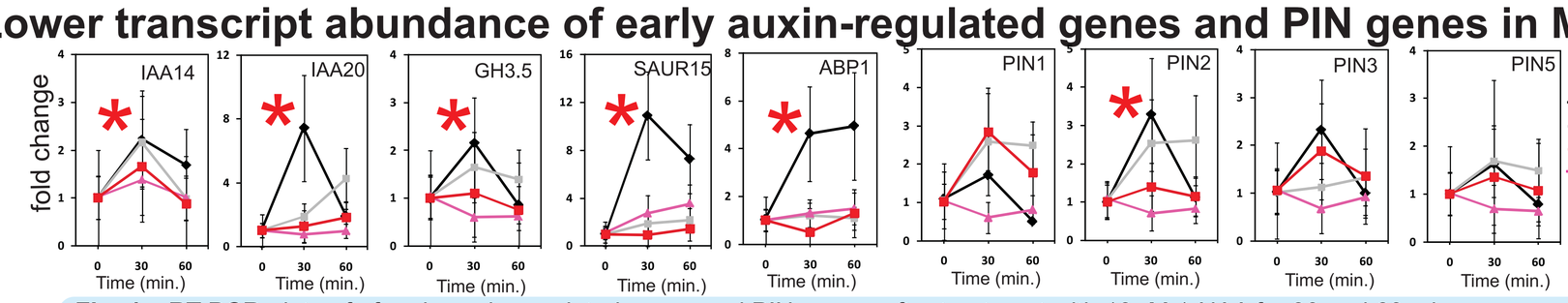
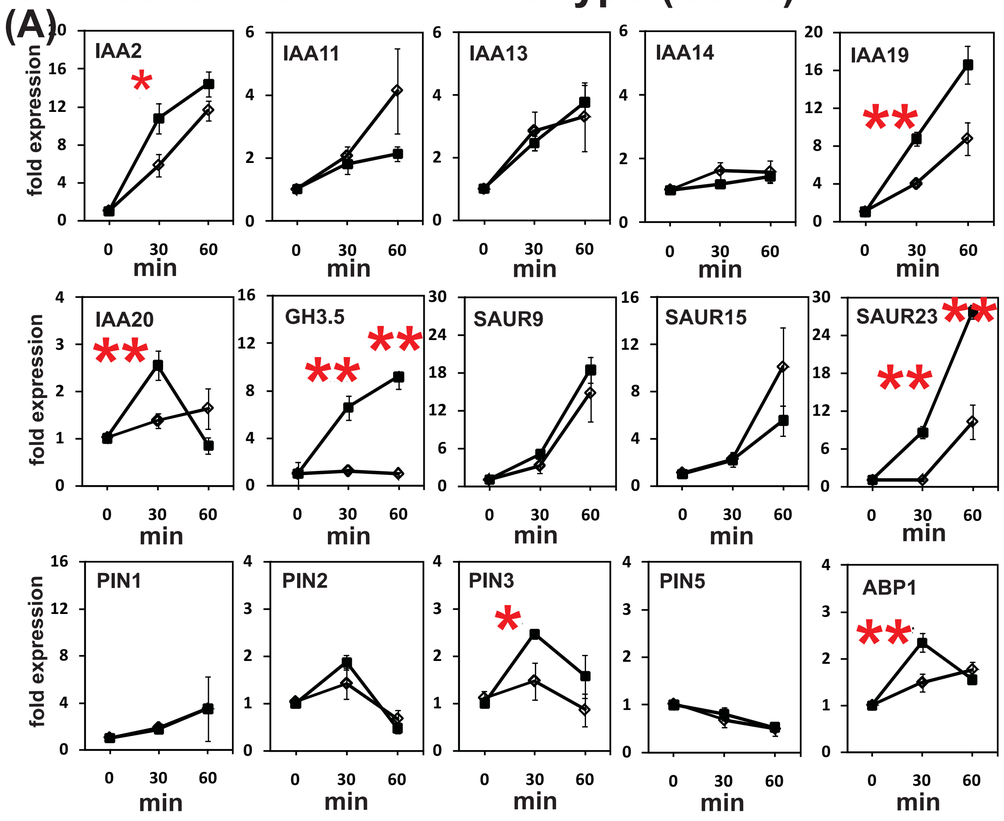


Fig. 4. qRT PCR data of of early auxin-regulated genes and PIN genes after treatment with 10µM 1-NAA for 30 and 60 min. M7 and M8 showed lower response in the expression of *GH3.5* and *PIN2* genes after 30 min auxin application in comparison to OEX ABP1 and wt. In comparison to wt only, most of the genes (*IAA14, IAA20, GH3.5, SAUR15, ABP1,* and *PIN2*) were lower regulated after 30 min 1-NAA application in M7 and M8. Data was collected from three biological replications and three technical replications of each.

Even a weak point mutant *abp1-5* showed slightly defects in auxin-regulated characters and lower response to auxin in regulation of early auxin-regulated genes

Transcriptional regulation of some early auxin-response genes and PIN genes in *abp1-5* mutant was slightly less auxin sensitive than in wild type (Col-0)



Phenotype appearance of *abp1-5* mutant

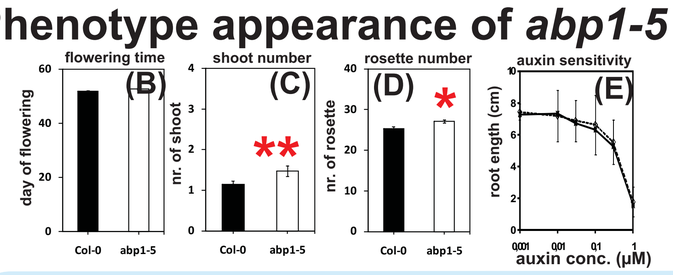


Fig. 5. Phenotypic appearance of 52 days old LD-grown plants. (A). Representative picture of 52 days old plants showed no difference in flowering time between *abp1-5* mutant and Col-0. (B) Graphic quantification of flowering time. Plants were grown under LD condition. (C) *abp1-5* mutant produced more shoots and rosette leaves (D) in comparison to Col-0. (E) No difference in root length was observed between *abp1-5* and Col-0 in response to different auxin concentrations.

Slightly defects in auxin-regulated phenotypes of *abp1-5* mutant

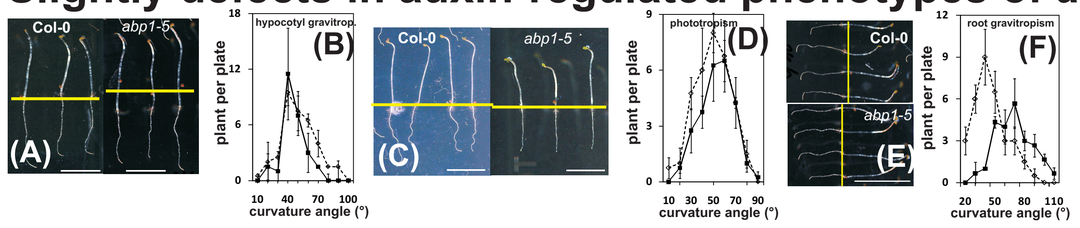


Fig. 6. Responses to gravity and light in *abp1-5* mutant seedlings (A) Representative images of a 3 d old seedlings after 24 gravitropic response and (B) their graphic quantification of hypocotyl angle showed no difference. (C) Representative image of 3 d dark-grown seedlings in responses to 8 h phototropism (10µmol $m^{-2}s^{-1}$ blue light) and (D) their quantification of hypocotyls bending which showed *abp1-5* produced smaller angles in comparison to Col-0. (E) Representative image of 3 d dark-grown seedlings after 24 h gravitropic response and (F) their grapic quantification which showed lower response in *abp1-5* in comparison to Col-0. Data were collected from three independent replications (n= 40-90 of each). Open diamonds: *abp1-5* mutant, black square: Col-0.

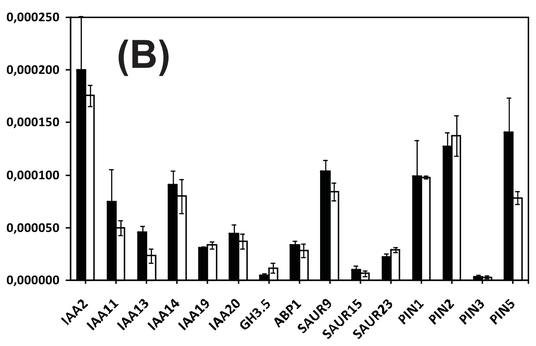


Fig. 7. Less auxin sensitive in transcriptional regulation of some early auxin-response genes and PIN genes in *abp1-5* mutant in comparison to wt in response to 10µM 1-NAA at 3 time points (0, 30, 60 min). A. Five genes (*IAA2, IAA19, IAA20, GH3.5, SAUR23*) of 10 early auxin-response genes were observed less regulated in *abp1-5* mutant in comparison to wt, whereas only *PIN3* of 4 PIN genes which was observed less regulated. Data was collected from 2-3 independent biological replication and 3 technical replication of each. Asterix indicates statistically significant ($p<0.001$ or $p<0.05$). Eventhough, basal level of transcriptional abundants the most of investigated-genes in *abp1-5* and wt were similiar, no statistic difference (B).

Conclusion: Our ABP1 point mutants, M7 and M8, showed defect in auxin-regulated phenotypes and low response to auxin in comparison to OEX-ABP1 and wt. A weak point mutant *abp1-5* also showed slightly similar response to auxin. All together it gives significant proves that a mutant of ABP1 will be a good tool for further researches in auxin physiology, especially auxin signaling.

Reference: **Effendi, Y., Rietz, S., Fischer, U., Scherer, GFE**. 2011. The heterozygous abp1/ABP1 insertional mutant has defects in functions requiring polar auxin transport and in regulation of early auxin-regulated genes. Plant. J 65: 282-294.

Effendi, Y and Scherer, GFE. 2011. Auxin Binding Protein1 (ABP1), a receptor to regulate auxin transport and early auxin genes in interlocing system with PIN protein and the receptor TIR1. Plant Signaling and Behaviour 6(8).

Presented on: International Conference in Botany: Botaniker Tagung 18-23 September 2011, Berlin Germany