Auxin-Binding Protein 1 (ABP1), the second auxin receptor

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ABSTRACT
Despite knowing 3-dimensional structure ABP1 is not fully acknowledged as an auxin receptor. We used the insertional ABP1 mutant (Chen et al., 2001). It is lethal when homozygous but viable in the hemizygous *abp1/ABP1* state. A hemizygous plant produces 2:1=resistant:wildtype progeny on kanamycin agar due to the T-DNAand this lethality. Seedlings form *abp1/ABP*1 plants are defect in phototropism and gravitropism of shoots and roots. Those populations are composed of a major slow reacting and a minor normal reacting group. *ABP1/abp1* seedlings show strong root slanting, longer hypocotyls, and slightly increased lateral root numbers. Root auxin responses (lateral root, main root length) in *abp1/ABP1* seedlings are slightly less sensitive than in wt. In short days and long days *abp1/ABP1* plants flower earlier. They have more branches and decreased main stem diameter, indicating decreased apical dominance. Auxin-induced genes (qPCR: *IAA2, IAA11, IAA13, IAA14, IAA19, IAA20, GH3.5, SAUR9, SAUR15, SAUR23*) respond to auxin (0.1uM/1uM) 2-15 fold stronger in WT than in *abp1/ABP1* seedlings (30 and 60 min). Thus, ABP1 is a receptor with probable functions in auxin transport and gene regulation. The apparent functional link to TIR1-link gene regulation could be provided by phospholipase A (Scherer *et al*., 2007, FEBS Lett.581:4205-4211).

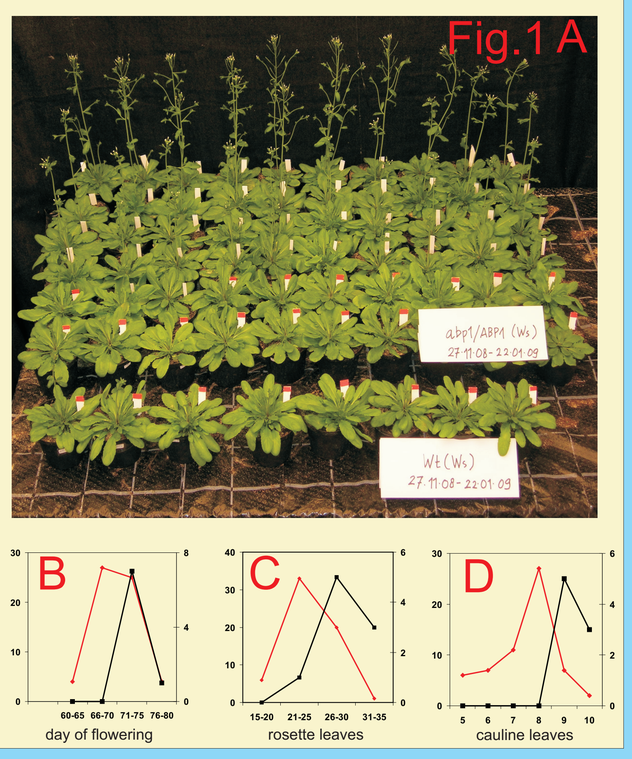


Fig.1. (A) Progeny of self mating of hemizygous *abp1/ABP1* produces a mixed population 2:1 = *abp1/ABP1*: Wt. (B) Hemizygous *abp1/ABP1* plants indicate early flowering (C) fewer rosette leaves and (D) fewer cauline leaves. Red line: hemizygous population, Black line: Wt only.

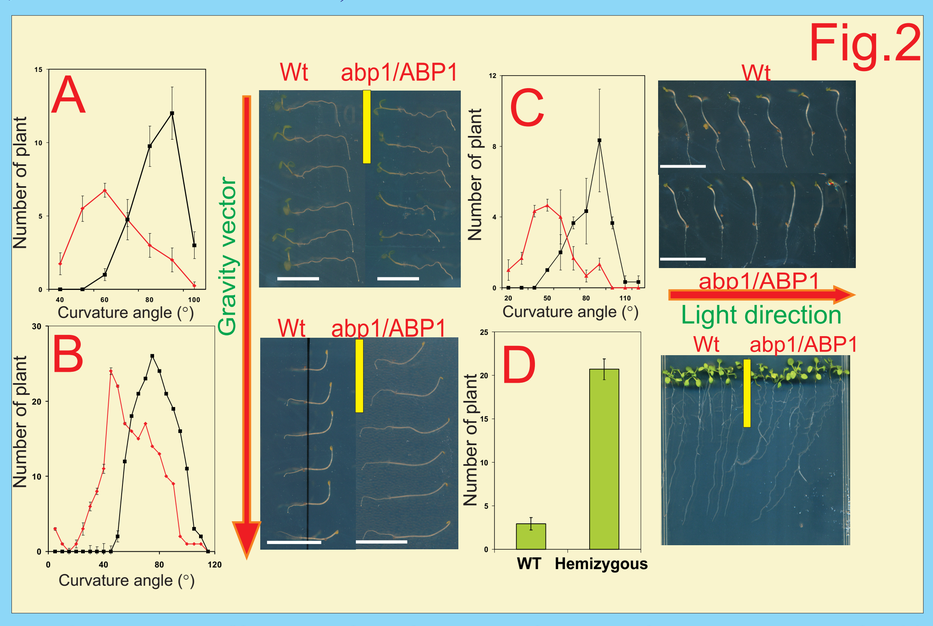


Fig. 2. Response of hypocotyls and roots to changing the gravity vector by 90° (A, B) and to lateral blue light (C). Progeny seedlings of a hemizygous *abp1/ABP1* plant are less responsive to 90° gravity in roots (A) and hypocotys (B) and to lateral blue light on hypocotyls (C) in comparison to Wt. Presence of wild type plants is indicated by a shoulder coinciding with the wild type curve. Agar plate in right side of each graphs show the bending of roots or hypocotyls. Red line: hemizygous *abp1/ABP1* in ratio 2:1, black line: Wt. (D) Hemizygous *abp1/ABP1* also show a root slanting angle (D).

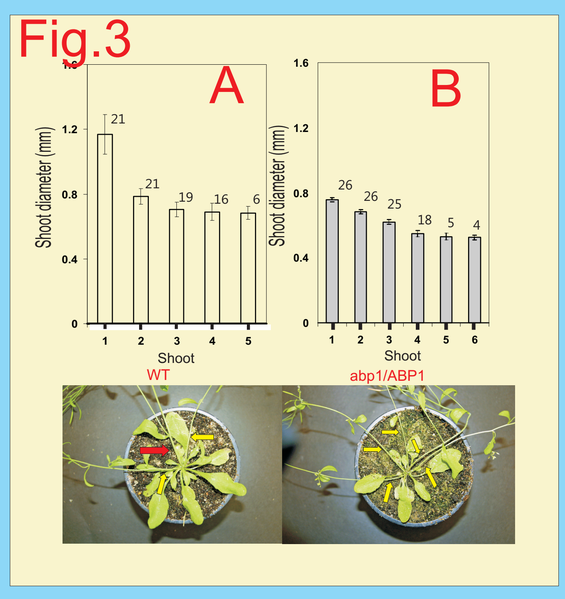


Fig. 3. Apical dominance is decreased in mutants. (A) Wt plants show one dominant shoot whereas (B) no dominant shoot in the diameters of hemizygous plants indicates that apical dominance is damaged. Figure left-below shows Wt plant which has one dominant shoot (red arrow) and left picture shows a hemiyzgous *abp1/ABP1* plant which shows no dominant shoot (yellow arrows).Plant were PCR-genotyped after growth.

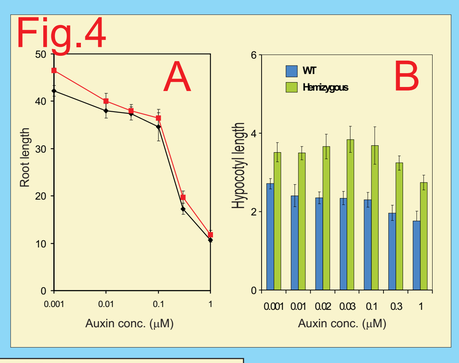


Fig. 4. Auxin sensitivity. Hemizygous *abp1/ABP1* seedlings have shorter hypocotyls in comparison to Wt in response to different levels of auxin con centrations (0.001mM, 0.01mM, 0.02mM, 0.03mM, 0,1mM, 0.3mM, 1mM) (B). However, no difference in root length between hemizygous *abp1/ABP1* and WT seedlings on the different levels of auxin application were found (A). Red line: hemiyzgous *abp1/ABP1*, black line: Wt. Growth on 0.5 MS agar and seedlings were selected for genotype.

Expression of early auxin-induced genes in hemizygous abp1/ABP1 and Wt in response to auxin application shows that they are less auxin-sensitive and reveals a link to TIR1

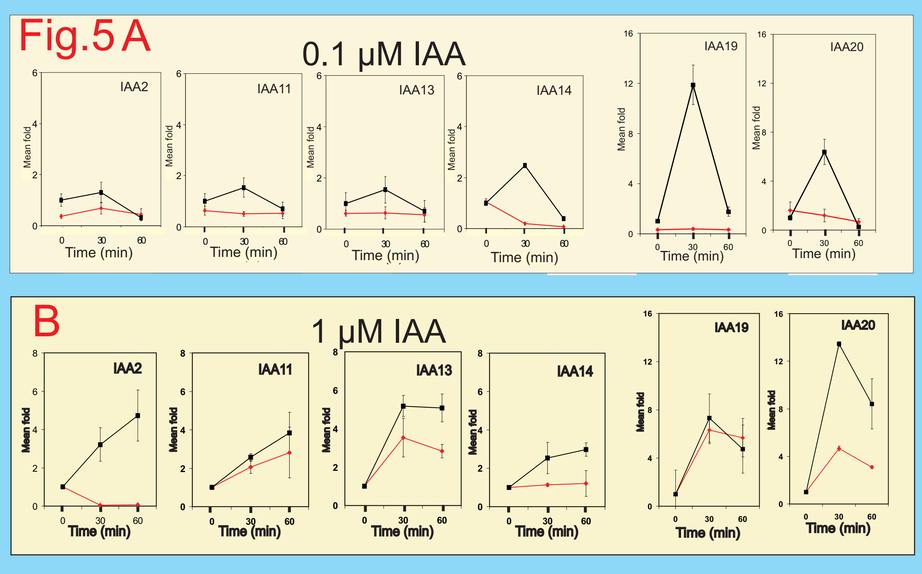
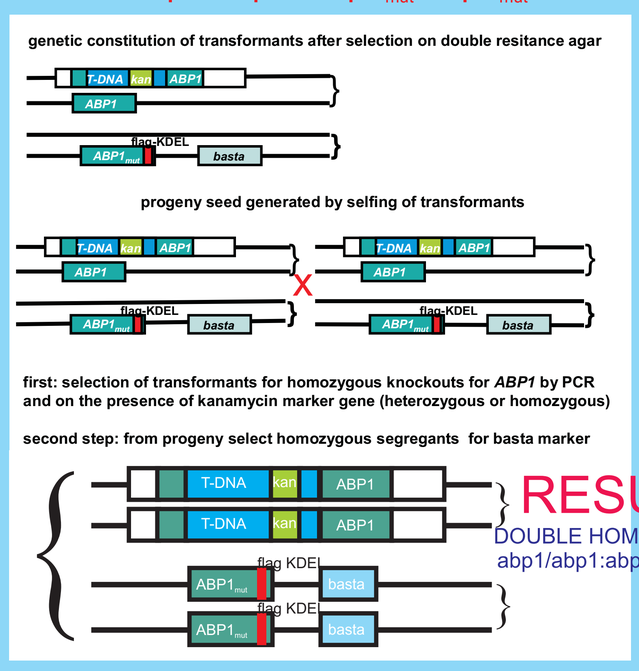


Fig. 5. Quantitative Real Time PCR data of early auxin-induced gene expression. (A) After 30 min 0.1mM auxin treatment, six IAA genes (*IAA2,IAA11,IAA13, IAA14, IAA19, IAA20*) showed increased expression in Wt samples but not in hemizygous *abp1/ABP1* samples. (B) With 1 mM auxin, after 30 min hemizygous *abp1/ABP1*and Wt samples showed increasing in gene expression in all IAA genes but Wt samples showed 1-10 fold more than hemizygous samples. (C) Increasing expression in another set of early auxin-induced genes is shown in fig. C . Three SAUR genes (*SAUR9, SAUR15, SAUR23), GH3.5* and the *ABP1* gene itself showed also 2-16 fold change different in Wt in comparison to hemizygous *abp1/ABP1* samples. Plants were selected on kan-agar, then grown for 5 d in liquid medium without kanamycin and treated with IAA.

Phenotypes of transformed $abp1_{mut}$ mutants containing mutated cDNAs (In the process of selection for eventually double homozygous abp1/abp1:$abp1_{mut}$/$abp1_{mut}$ genetic constitution)

Schema of design of double homozygous abp1/abp1: $abp1_{mut}$/$abp1_{mut}$



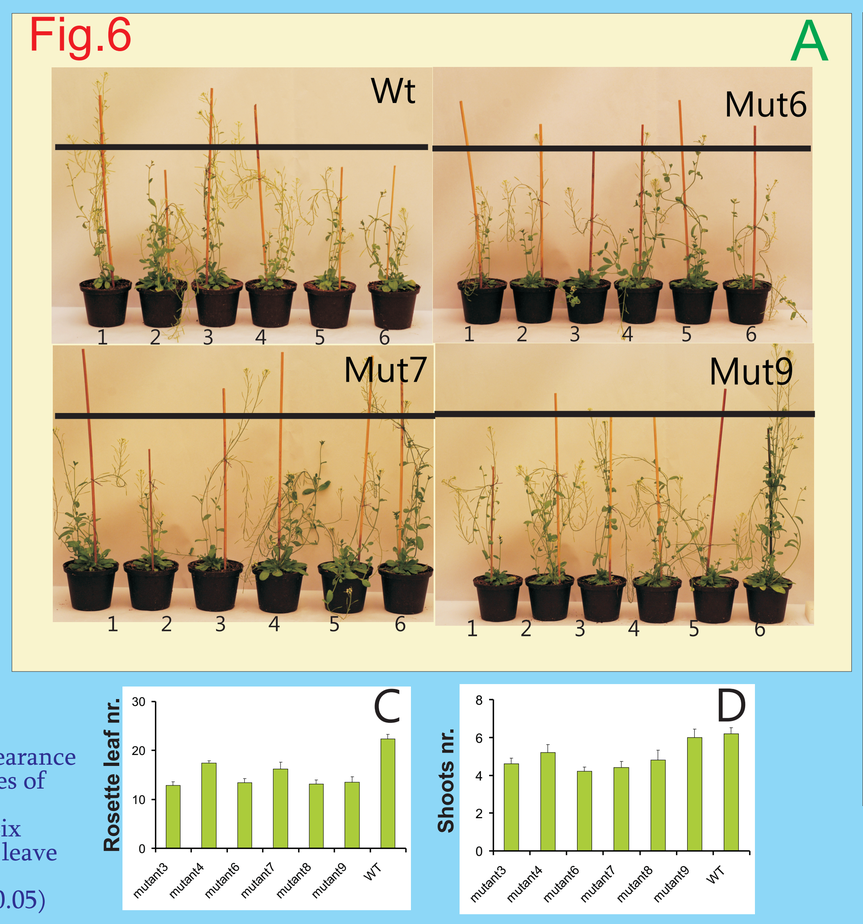


Fig. 6. Features of 80 day old mutant plants growth in LD. General appearance of mutans and Wt plants are shown on fig. A. Fig. B shows detail pictures of six plants of each mutants. Yellow arrows show curly leaves which are reminiscent of *phot1phot2* mutant (see phototropic deficiency above!). Six different mutants were investigated and all mutants show fewer rosette leave numbers in comparison to Wt ($p < 0.05$) (C), indicating early flowering. They also had reduced shoot numbers, especially in mutant 6 and 7 ($p<0.05$) (D), indicating lower apical dominance.

phot1phot2 plants show curly leaves

Conclusion: ABP1 is a powerful receptor which regulates genes, likely with a functional link to TIR1. The hemizygous abp1/ABP1 mutant is defect in responses requiring polar auxin transport. Early flowering in SD and LD is another property. Our in vitro mutant plants seem to have similar phenotypes.

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