MIS-REGULATION OF EARLY AUXIN-INDUCED GENES IN PHOSPHOLIPASE A KNOCKOUTS

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Patatin-related phospholipase A are coded by ten genes (AtPLAs) in Arabdipsis thaliana and are involved in auxin and pathogen signaling (e.g. Rietz et al., 2010, Mol. Plant). Here we used the T-DNA insertion mutants of the AtPLAIIIA, AtPLAIVB, AtPLAIVC, AtPLAIVD and AtPLAV to test the regulation of early auxin genes. Test genes were IAA2, IAA11, IAA13, IAA20, SAUR9, SAUR15, SAUR23, GH3.5 and genes involved in lateral root formation (IAA1, IAA4, IAA9, PIN3). 30 to 60% of the genes tested failed to respond to auxin (1 µM/10 µM) at t=30 min in the different knockouts. In most mutants the genes IAA11, IAA13 and IAA20 showed no change in gene expression in comparison to the WT, except IAA2 in AtPLAVB and AtPLAVD. Many of the genes involved in lateral root formation and the SAUR genes showed s atrong defect in gene expression in the mutants after auxin application. The mutants did not show any phenotypes under normal growth conditions. Only AtPLAIVA showed 50% lateral root formation on low nutrient medium. AtPLAIVC reacted less sensitive to ABA and to Phosphate deficiency (Riets et al, 2010). Because the abp1/ABP1 mutant regulated none of early auxin-induced genes properly at 30 min (see poster Effendi et al) we hypothesize that ABP1 and PLAs act in the same auxin signaling pathway influencing TIR1 activity in an unknown way (FEBS Lett 581: 4205-4211)

T-DNA insertion mutants of AtPLA

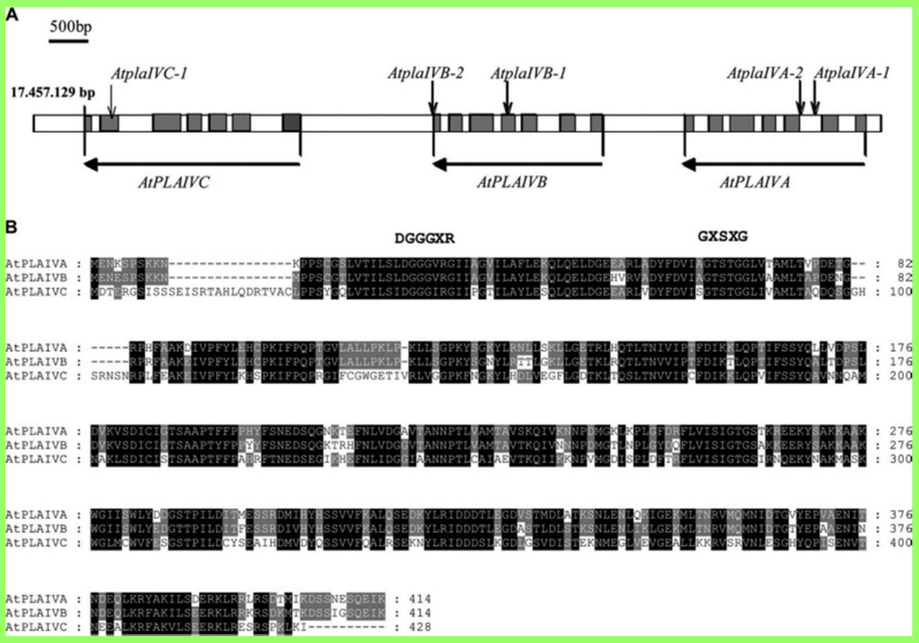


Fig.1.Tandem Gene Structure and protein Sequence Alignment of *AtPLAIVC* (At4g37050), *AtPLAIVB* (At4g37060), and *AtPLAIVA* (At4g37070).

Growth Analysis of *AtplaIVC*

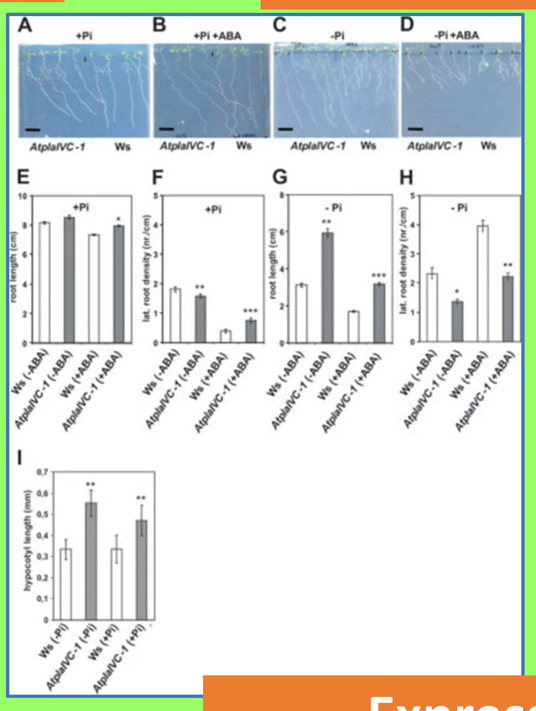
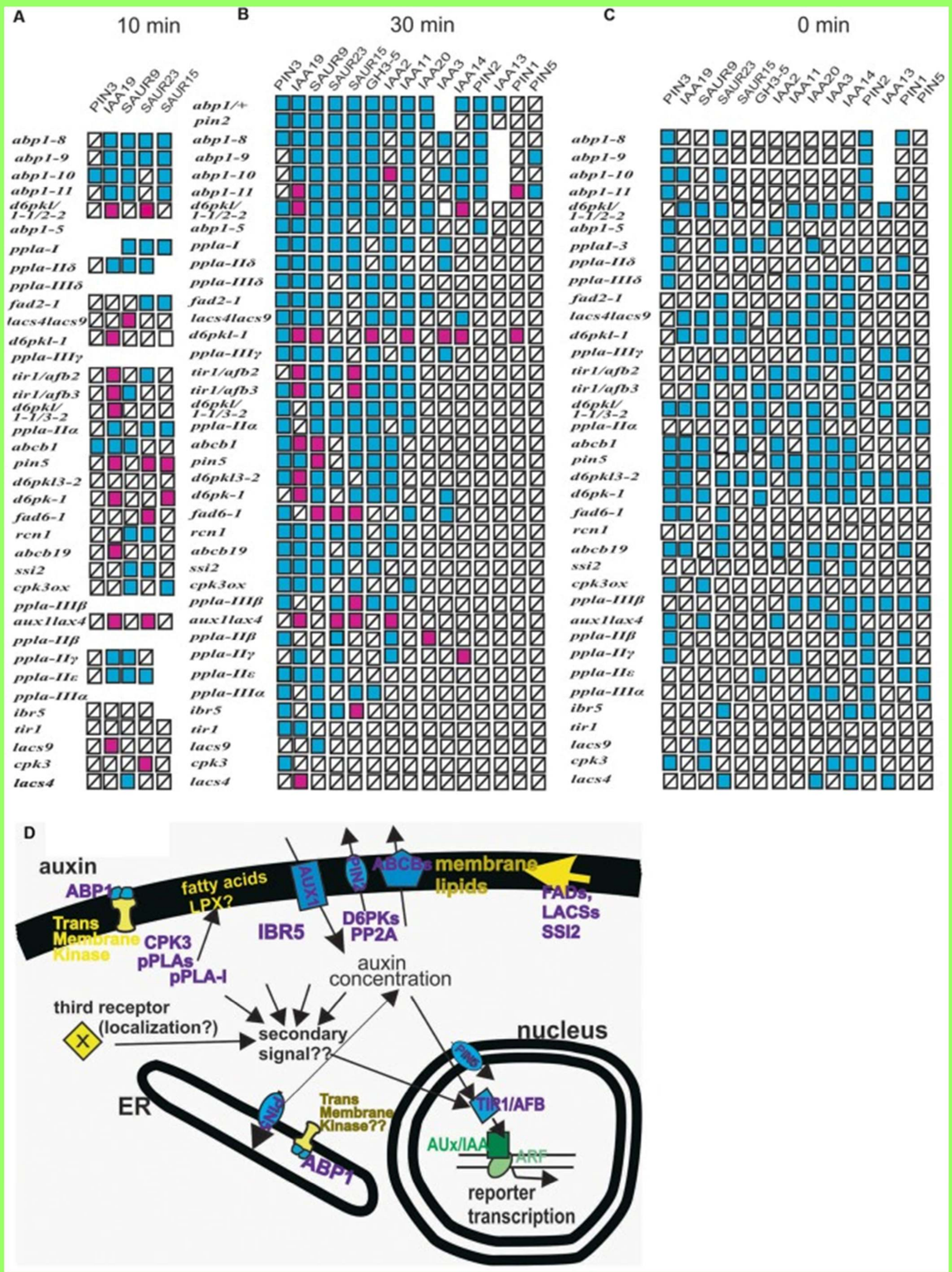
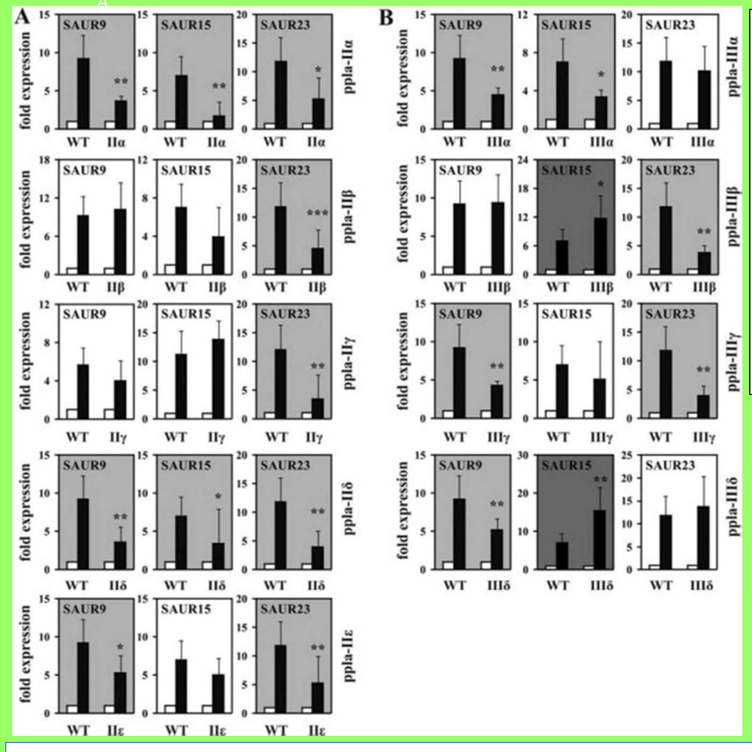


Fig. 2. (A–D) Seedlings grown on B5 (1:50) minimal diluted medium (bar = 1 cm). Stars above columns indicate significant differences between *AtplaIVC-1* and the corresponding Ws treatments at $P < 0.05$ (*), $P < 0.001$ (**), and $P < 0.0001$ (***) level following Student's *t*-test. (A) Medium supplemented with 1 mM $KHPO_4$. (B) Medium supplemented with 1 mM $KHPO_4$ + 0.6 µM ABA. (C) No addition. (D) Medium supplemented with 0.6 µM ABA only.
(E) Primary root lengths quantified from (A) and (B).
(F) Lateral root density quantified from (A) and (B).
(G) Primary root lengths quantified from (C) and (D).
(H) Lateral root density quantified from (C) and (D).
(I) Hypocotyl length quantified from (A) and (C) (n = 15–28; $P < 0.001$ for each relevant pair).

Summary of auxin-induced transcription in mutants and the number of defects in early auxin-induced gene expression



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



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.

Fig. 4. Defects in early auxin induced genes expression in auxin-related mutants (A-C). Red squares represent increased transcription as compared to the wt and blue squares represent decreased values than those found in the wt. Squares with a black bar represent transcription not significantly different from the wt. Previously published data are incorporated into the figure (Effendi and Scherer, 2011; Effendi et al., 2011, 2013, 2014; Labusch et al., 2013). To allow for a synopsis and a comparative analysis, we assembled all previously published results on *ppla* and *abp1* mutants (Effendi et al., 2013, 2014, 2015; Labusch et al., 2013) and the experiments here into one scheme. The mis-expression of the reporter genes in plants after 10 min of auxin treatment, after 30 min of 10 µM auxin treatment , and at t = 0 min are presented.
(D) The mutant gene products are arranged into a schematic cell to indicate their subcellular localization. A hypothetical linkage between TIR1 and ABP1 is indicated by arrows. Also, the possibility of a hypothetical third receptor with unknown localization is indicated.

CONCLUSION

The T-DNA PLA knockouts mutants showed mis-regulation of early auxin regulated genes. The some other auxin related mutants also showed early auxin regulated genes in various level. Since abp1 mutants has been showed as auxin rceptor for auxin-early induced genes, then we hypothesizes that ABP1 and PLAs act in the same auxin signaling pathway .