

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

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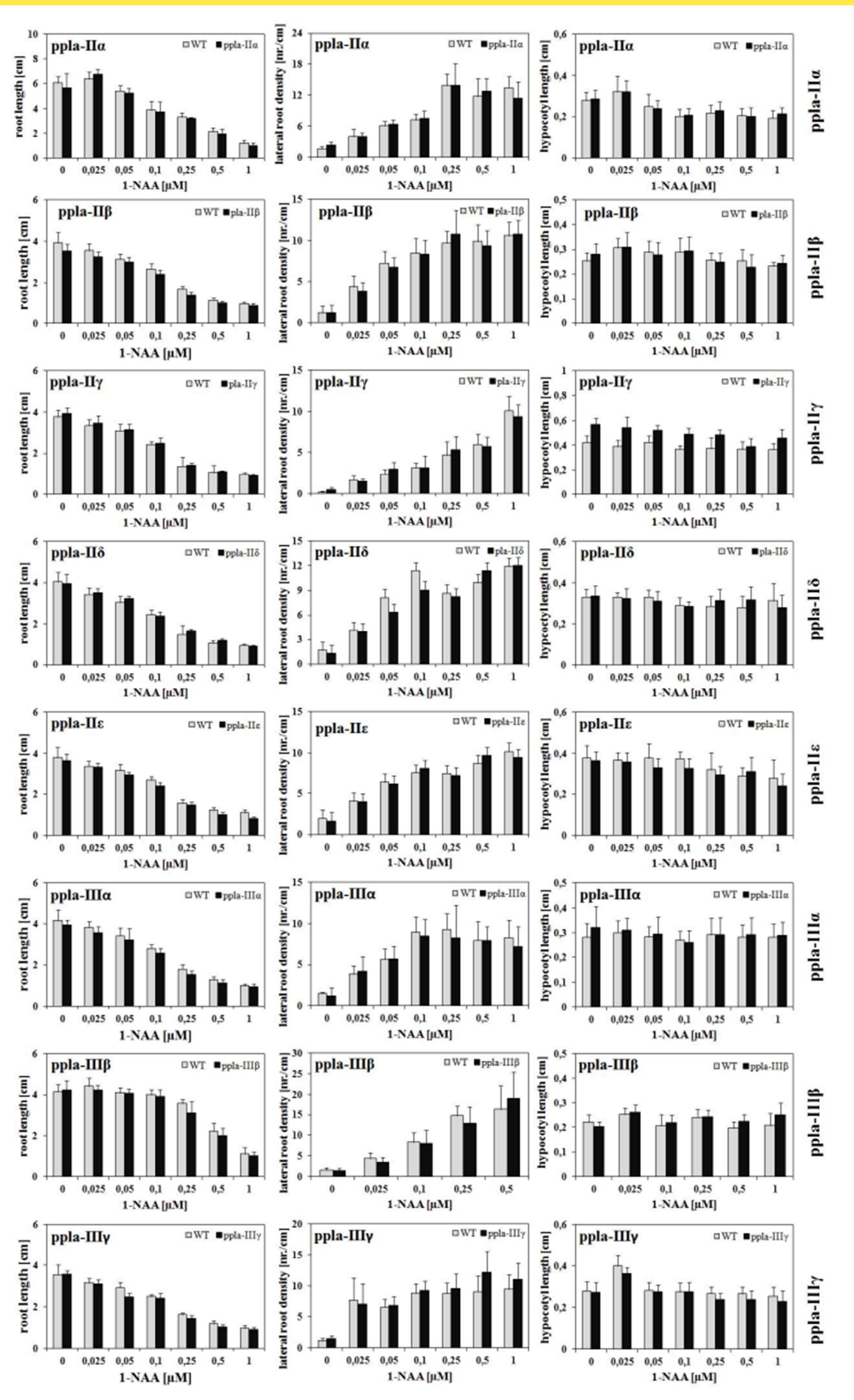
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In Arabidopsis, a family of ten phospholipase A genes has been identified and are involved in auxin and pathogen signaling (Rietz et al, 2010, Mol. Plant). Plant PLA activity is rapidly induced by different external signals and the PLA reaction products 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 disappeared after 3 hours. Because the *abp1/ABP1* mutant regulated of none 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.)

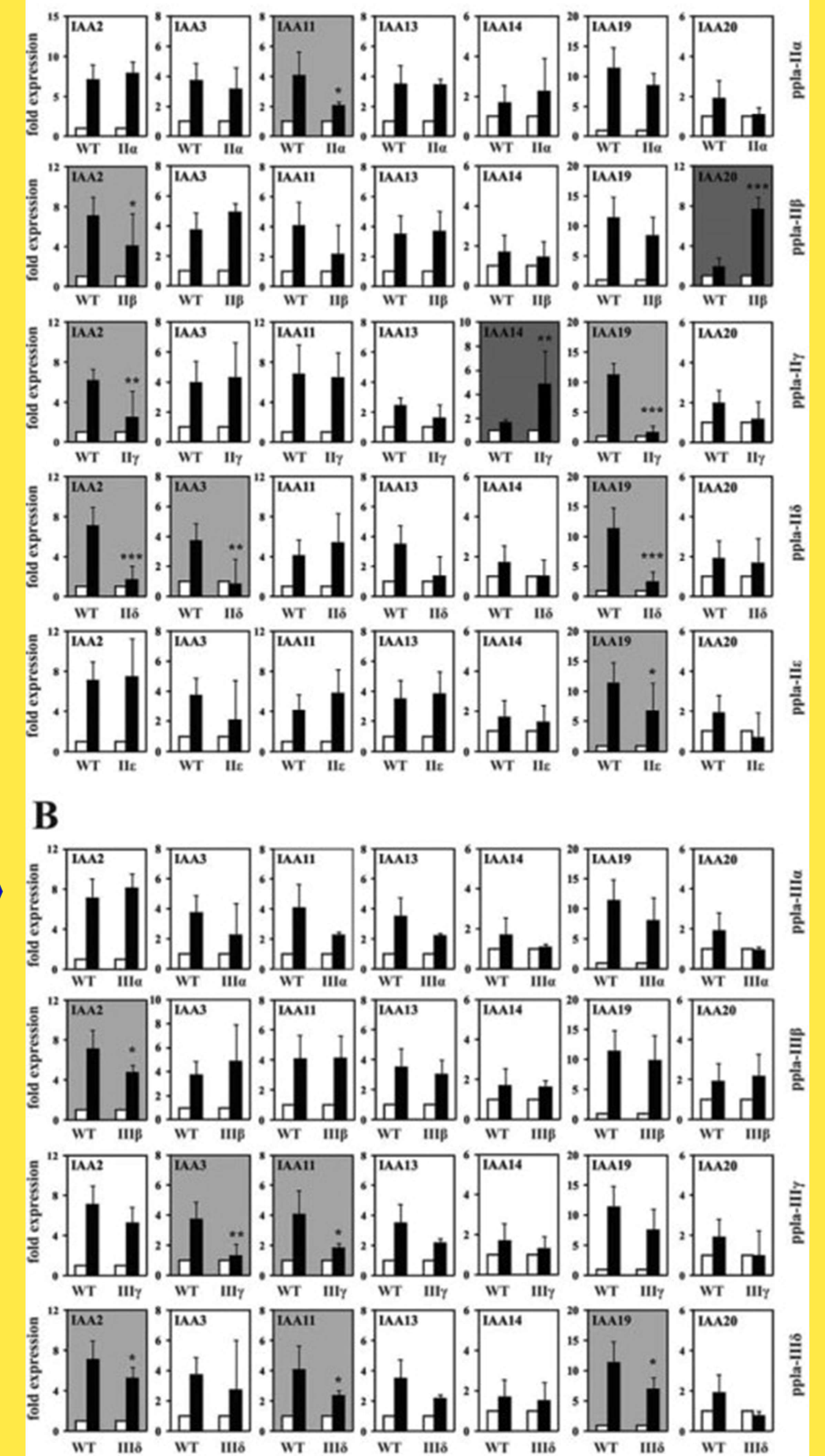
Physiological respond of eight T-DNA insertion *pplA* mutants in response to auxin application



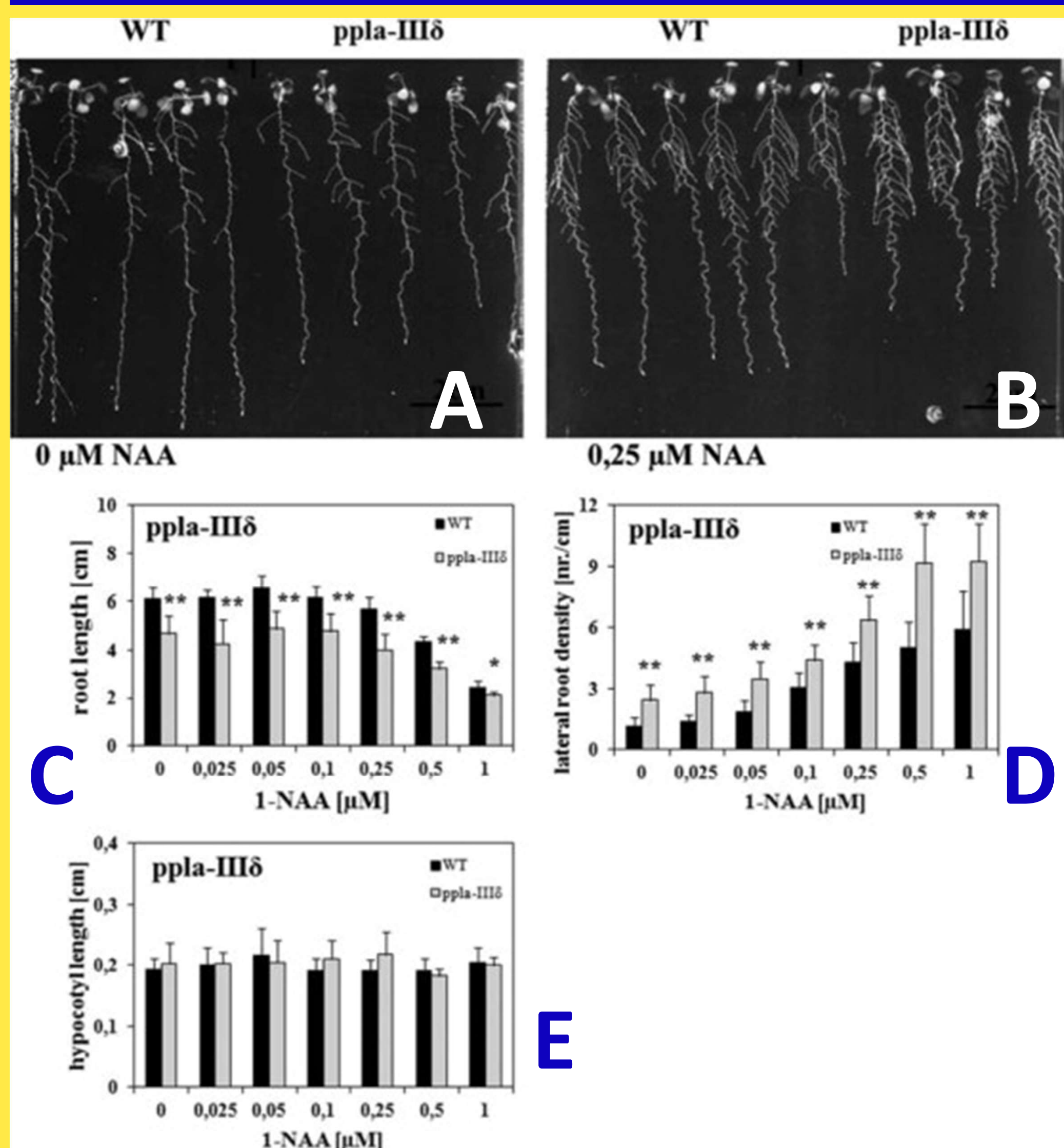
Physiological responses to auxin of T-DNA insertion mutants in root and hypocotyl length, and lateral root density. Plants were grown in white light for 7 d on upright agar plates in the presence of increasing concentrations of 1-NAA. None of *pplA* mutants showed an auxin phenotype, except *pplA-IIIδ*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

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 $t = 0$ min to 1 (white bars); values at $t = 30$ min IAA were calculated accordingly (black bars).

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



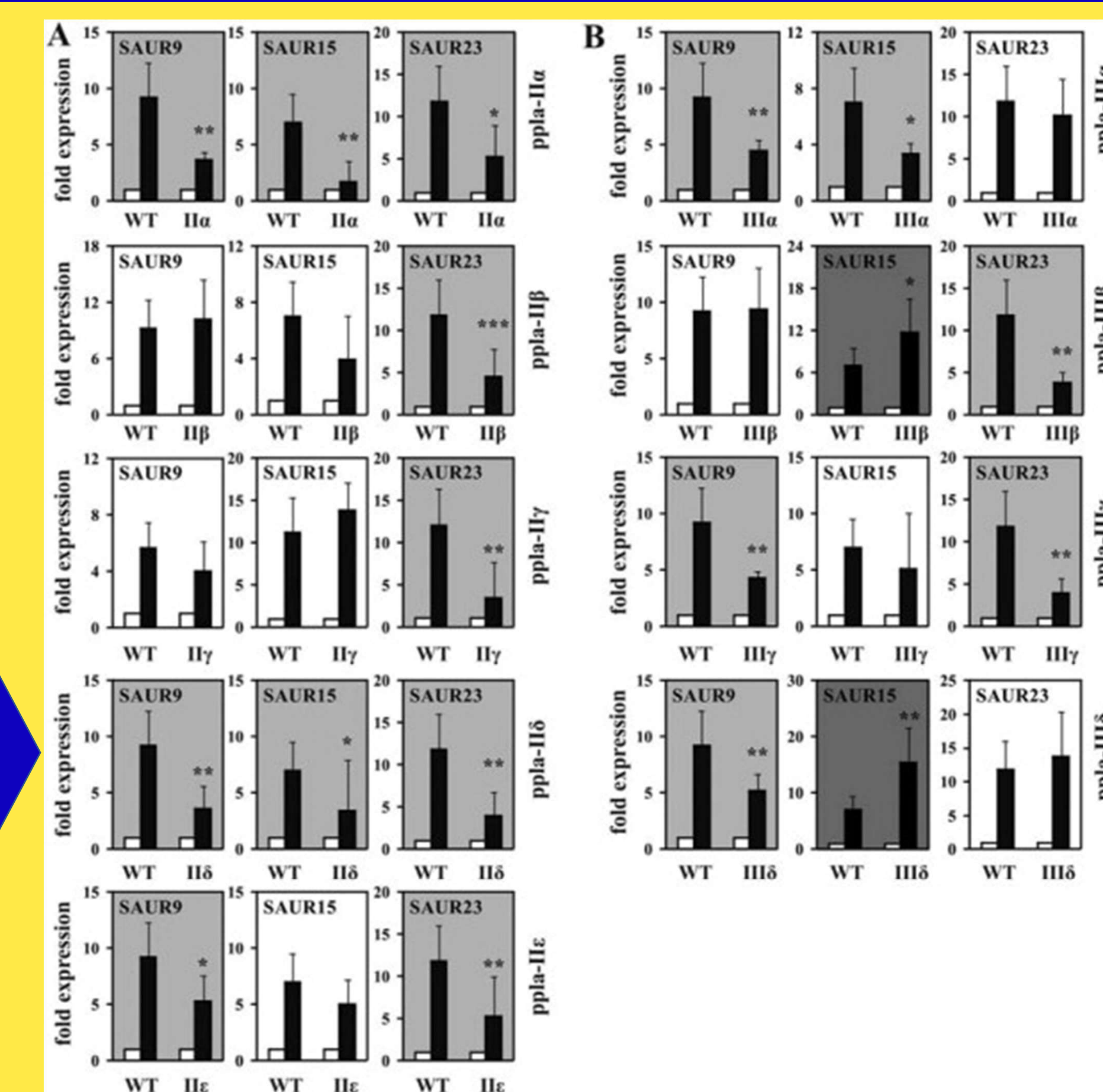
Growth Response of Light-Grown *pplA-IIIδ* Mutants and Wild-Type Plants in Response to Auxin.



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 disappeared after 3 hours. Because the *abp1/ABP1* mutant regulated of none 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.)