ATPLA-I A PHOSPHOLIPASE A WITH FUNCTIONS IN GRAVITROPISM, PHOTOTROPISM AND ROOT TIP MOVEMENT

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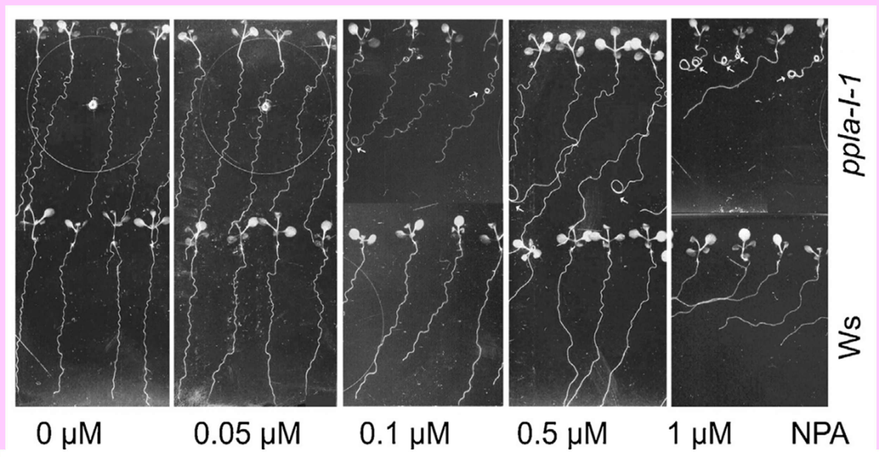
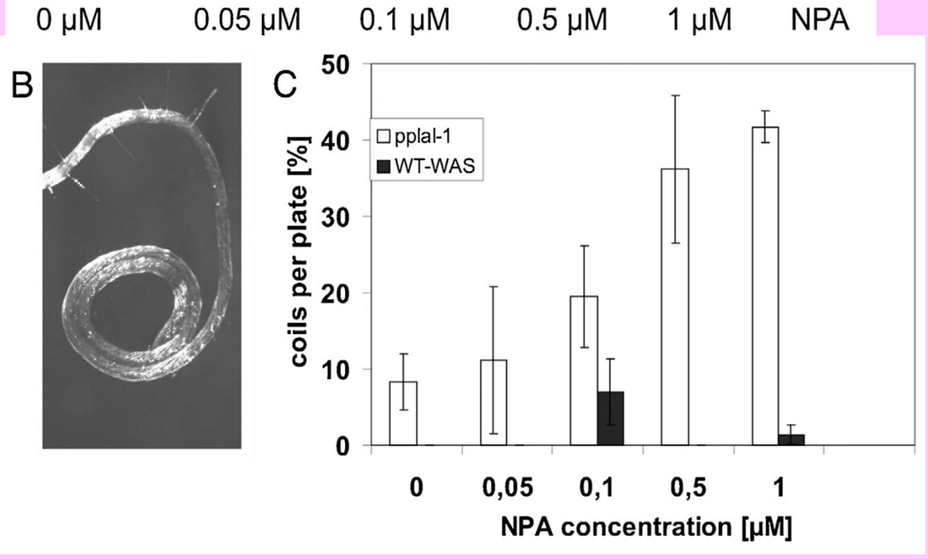
Phospholipase A enzymes cleave phospho- and galactolipids to generate free fatty acids and lysolipids that function in animal and plant hormone signaling. Here, we describe three *Arabidopsis patatin-related phospholipase A* (*pPLA*) genes *AtPLAIVA*, *AtPLAIVB*, and *AtPLAIVC* and their corresponding proteins. Loss-of-function mutants reveal roles for these pPLAs in roots during normal development and under phosphate deprivation. *AtPLAIVA* is expressed strongly and exclusively in roots and *AtplaIVA*-null mutants have reduced lateral root development, characteristic of an impaired auxin response. By contrast, *AtPLAIVB* is expressed weakly in roots, cotyledons, and leaves but is transcriptionally induced by auxin, although *AtplaIVB* mutants develop normally. *AtPLAIVC* is expressed in the floral gynaecium and is induced by abscisic acid (ABA) or phosphate deficiency in roots. While an *AtplaIVC-1* loss-of-function mutant displays ABA responsiveness, it exhibits an impaired response to phosphate deficiency during root development. Recombinant AtPLA proteins hydrolyze preferentially galactolipids and, less efficiently, phospholipids, although these enzymes are not localized in chloroplasts. We find that AtPLAIVA and AtPLAIVB are phosphorylated by calcium-dependent protein kinases *in vitro* and this enhances their activities on phosphatidylcholine but not on phosphatidylglycerol. Taken together, the data reveal novel functions of pPLAs in root development with individual roles at the interface between phosphate deficiency and auxin signaling.

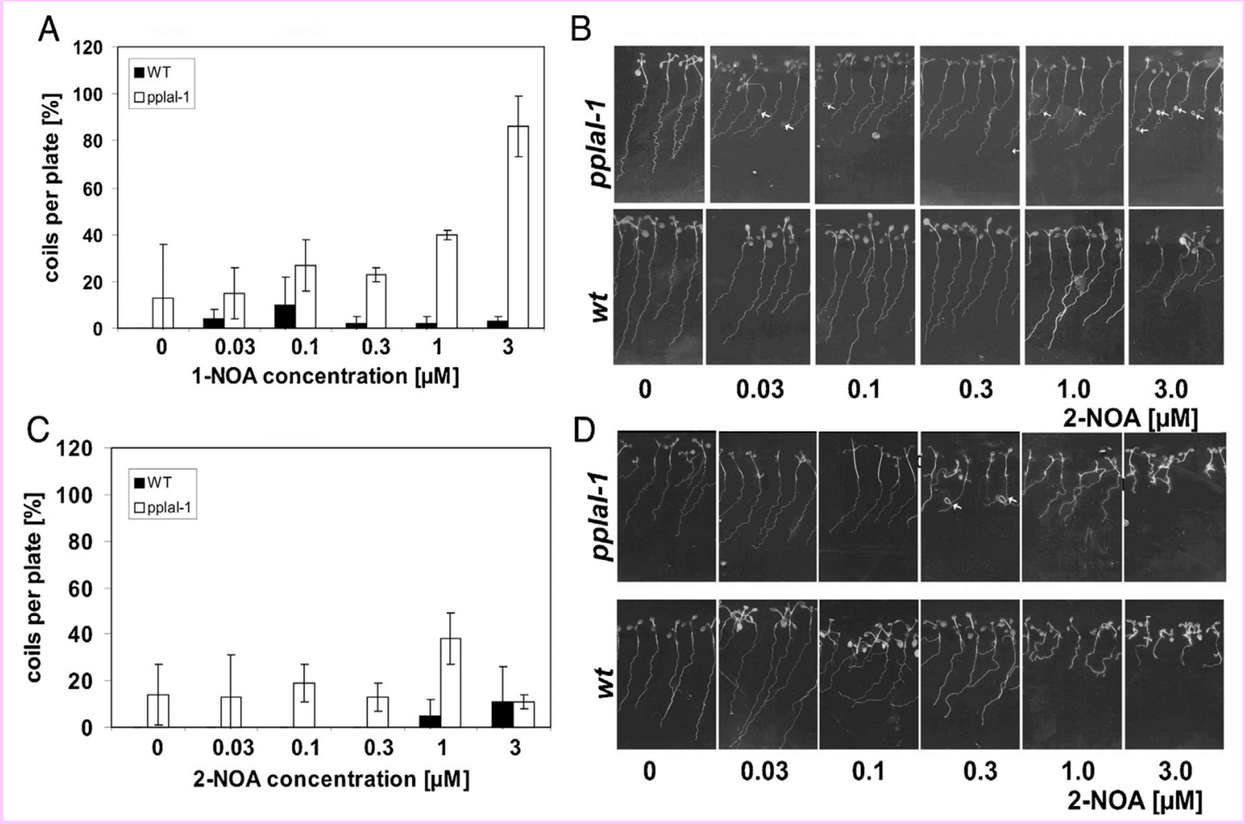
Defect in auxin response induces root coiling in *AtPLAI-I* mutants





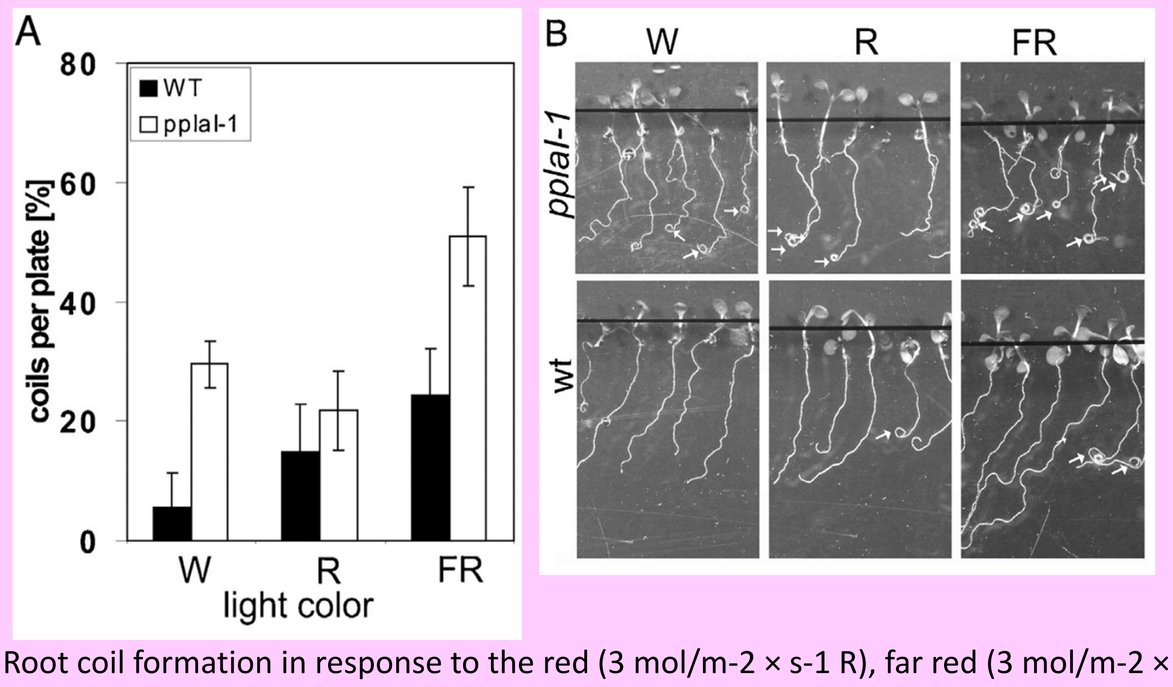
Root coil formation in response to the auxin efflux inhibitor NPA. (A) Scans of seedlings. Arrows indicate coils. (B) Single coil from the mutant pplaI-1. (C) Quantification of coils. After three days of growth on upright plates coiling was induced by inclining the plates back by 45◦ relative to the horizontal surface and then keeping them 10 further days supplemented with NPA as indicated. Values are averages of six plates with 36–48 seedlings per concentration; S.E.

Application of auxin inhibitor 1-NOA dan 2-NOA show no reduction on root coiling formation in *AtPLAI-I* mutants



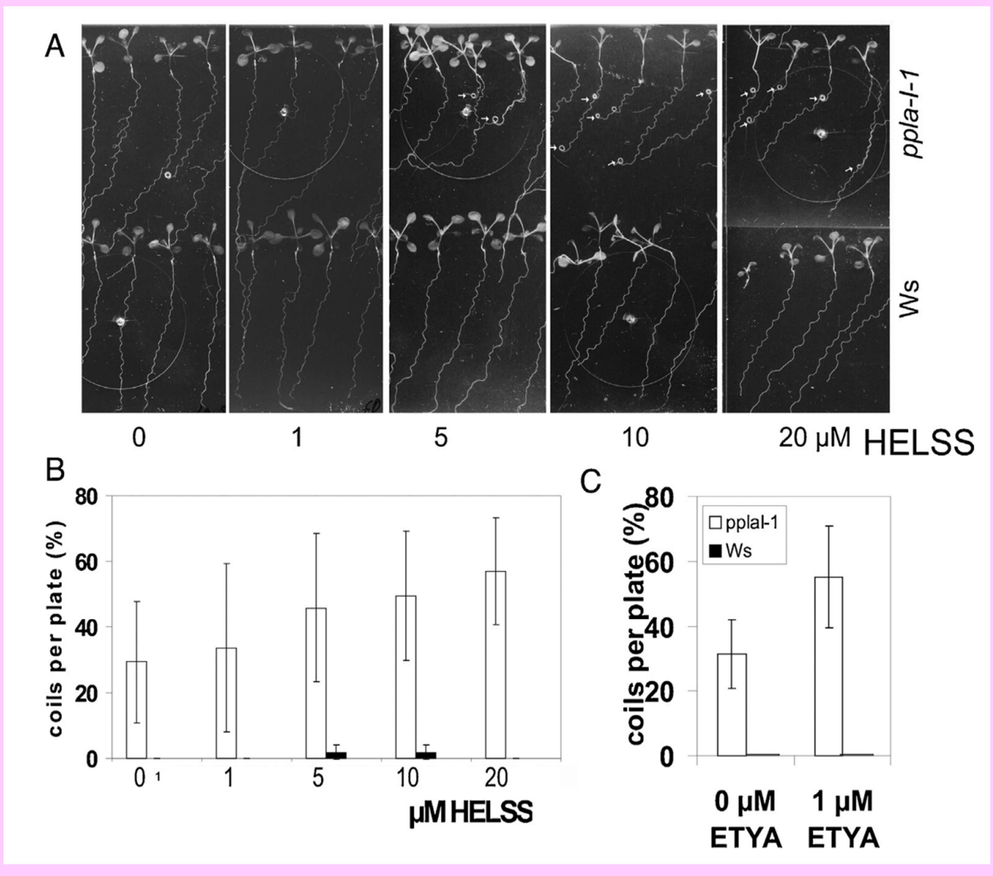
Root coil formation in response to the auxin influx inhibitor 1-NOA and the analog on 2-NOA. (A, C) Quantification of coils. (B, D) Scans of seedlings. Arrows indicate coils. After three days of growth on upright plates coiling was induced by inclining the plates back by 45◦ relative to the horizontal surface and then keeping them 10 further days supplemented with inhibitors as indicated. Values are averages of six plates with 36–48 seedlings per concentration; S.E.

Far-Red, Red and White Lights induce more root coilings in *AtPLAI-I* mutants



Root coil formation in response to the red (3 mol/m-2 × s-1 R), far red (3 mol/m-2 × s-1 FR) and white (50 mol/m-2 × s-1 W) light. (A) Quantification of coils. (B) Scans of seedlings. Arrows indicate coils. After three days of growth on upright plates coiling was induced by inclining the plates back by 45◦ relative to the horizontal surface and then keeping them 10 further days in different light conditions as indicated. Values are averages of six plates with 36–48 seedlings per concentration; S.E

Auxin and Phospholipase inhibitors induce root coiling formation in *AtPLAI-I* mutants



Root coil formation in response to the auxin and phospholipase inhibitors HELSS and EYTA. (A) Scans of seedlings. Arrows indicate coils. (B, C) Quantification of coils. After three days of growth on upright plates coiling was induced by inclining the plates back by 45◦ relative to the horizontal surface and then keeping them 10 further days supplemented with inhibitors as indicated. Values are averages of six plates with 36–48 seedlings per concentration; S.E