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Functional Plant Biology

Supplementary Material

PLDα1 and GPA1 are involved in the stomatal closure induced by Oridonin in *Arabidopsis thaliana*

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Effects of different concentrations of Oridonin on *A. thaliana* seedlings

The growth status of *A. thaliana* seedlings treated with different concentrations of Oridonin for five days was shown in Supplementary Fig. 1. The seedlings were growing normally and the leaves were green and spreading after 0–70 μmol·L$^{–1}$ Oridonin treatment. But after 80 μmol·L$^{–1}$ Oridonin, the leaves gradually turned yellow. And the leaves were yellowing and crimping after 100 μmol·L$^{–1}$ Oridonin treatment. These results reflected that the high concentration of Oridonin could inhibit the growth of *A. thaliana* seedlings.

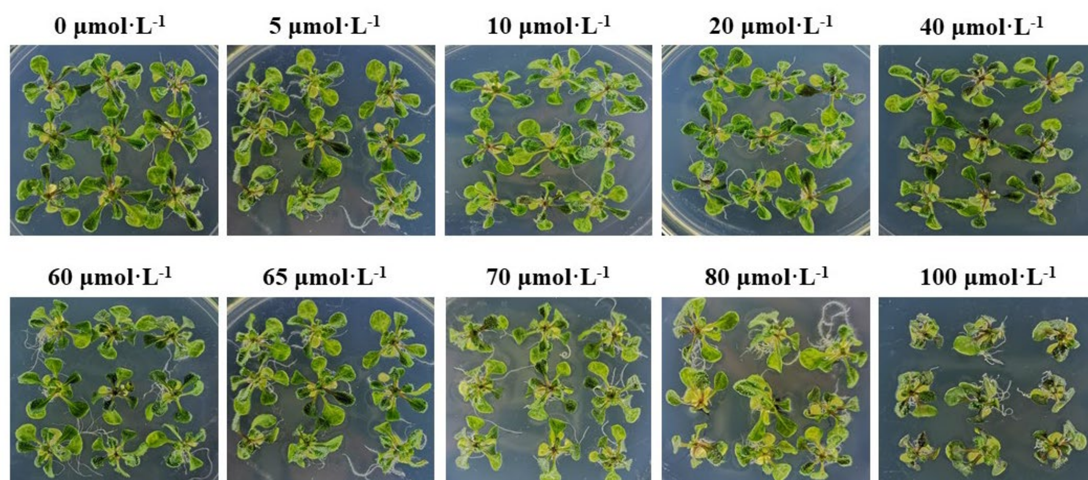

Fig. S1. Effects of different concentrations of Oridonin on the growth of *A. thaliana* seedlings. 16-day-old *A. thaliana* seedlings were treated with Oridonin for 5 days.

Identification of *pldα1*, *gpa1*, and *pldα1/gpa1* mutants

The *A. thaliana pldα1* (SALK_067533) and *gpa1* (CS_6534) mutants needed for the experiment were obtained from the *A. thaliana* Biological Resource Center (ABRC). With WT as control, the mutant was identified by the 'three-primer method'. After 14 days of growth, the seedling leaves of *pldα1* and *gpa1* were cut and genomic DNA was extracted as a template. Two pairs of primers (AtPLDα1LP and AtPLDα1RP, and AtGPA1LP and AtGPA1RP) were respectively used for PCR amplification. WT can be amplified by gene-specific primers (LP+RP). The homozygous *pldα1* and *gpa1* can be amplified with T-DNA-specific primers (LBB1.3+RP) (Fig. 2A). In subsequent experiments, using the hybridized *pldα1* and *gpa1* as the parents, the *pldα1*/*gpa1* double mutants were identified and screened (Fig. 2B). The gene expression levels of *PLDα1* and *GPA1* in *pldα1* and *gpa1* mutants were determined by real-time fluorescence quantitative PCR (RT-qPCR) (Fig. 2C, D). Compared with the WT, the gene relative expression levels of *PLDα1* and *GPA1* were weak in *pldα1* and *gpa1*, respectively. And the relative gene expression levels of *PLDα1* and *GPA1* in *pldα1*/*gpa1* were also very weak. The PLD activity and GTP hydrolase activity in *pldα1*, *gpa1*, and *pldα1*/*gpa1* mutants were significantly lower than those in the WT. As a result, the mutant *pldα1*/*gpa1* was successfully constructed and could be used in subsequent experiments.

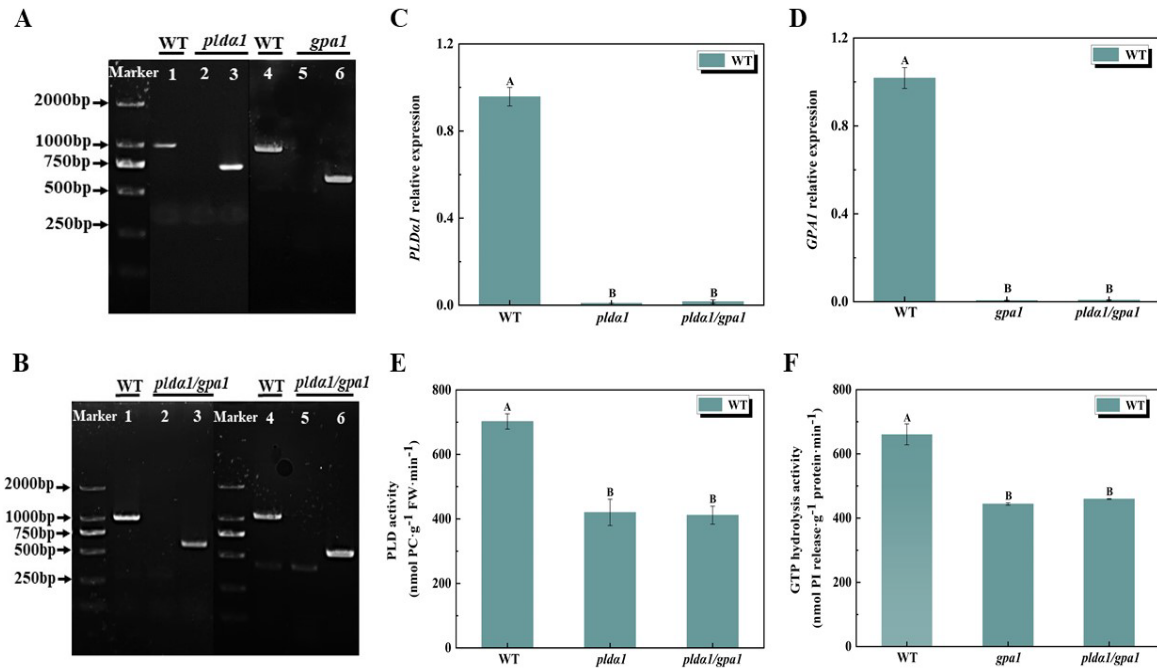

Fig. S2. Identification of *pldα1*/*gpa1* double mutant. (A) Molecular analysis of WT, *pldα1*, and *gpa1* primers LP, RP, and LB (LBb1.3) were used to target the flanking sequences of the T-DNA. (B) Molecular analysis of WT and *pldα1/gpa1*. Lane 1: WT + AtPLDα1LP + AtPLDα1RP, lane 2: template + AtPLDα1LP + AtPLDα1RP, lane 3: template + LBb1.3 + AtPLDα1RP. Lane 4: WT + AtGPA1LP + AtGPA1RP, lane 5: template + AtGPA1LP + AtGPA1RP, lane 6: template + LBb1.3 + AtGPA1RP. (C-D) RT-qPCR analysis of *PLDα1* and *GPA1* relative expressions in WT, *pldα1*, *gpa1*, and *pldα1/gpa1*. (E-F) The PLD and GTP hydrolysis activity in WT, *pldα1*, *gpa1*, and *pldα1/gpa1*. Note: Values were the average of three biological repeated experiments, means with different letters denote statistically significant differences at $P < 0.05$, according to one-way ANOVA. Capital letters indicate significant differences between groups.