SUPPLEMENTARY MATERIALS

(Zhang et al., 2018)

SUPPLEMENTAL METHODS

**Soluble carbohydrates measurement in seedlings**
Soluble carbohydrates were determined as described by Vergauwen et al. (2000) with some modifications. Ten-day old Arabidopsis seedlings, grown on ½MS plates without sucrose, were separated in shoot- and root parts and immediately frozen in liquid nitrogen. Samples were ground and their fresh weights recorded for further analysis. Soluble sugars were extracted in water and immediately boiled in a water bath. Carbohydrates were separated by anion-exchange chromatography and quantified by pulsed-amperometric detection (Dionex, Sunnyvale Ca, USA). Mannitol was used as an internal standard. Factors for Glc, Fru, Suc were obtained by injecting pure compounds. A Carbopac™ PA-100 guard and Carbopac™ PA-100 (4 x 250) in series were equilibrated with 90 mM NaOH for 24 min. Regeneration was 5 min with 500 mM Na-acetate and 10 min with 500 mM NaOH. Values shown are the means ± SD (n=3) for one representative experiment that was repeated twice.

**Phloem sap soluble carbohydrates measurement**
Phloem exudates were extracted and analyzed as described earlier (Roessner et al., 2000; Tetyuk et al., 2013). The hydrophilic fraction (sugars, amino acids, small molecules) was dried and derivatized using methoxyamine in pyridine and BSTFA (N,O-bis-trimethyl-silylfluoroacetamide) with 1% trimethylchlorosilane. The metabolites were subsequently separated using an Agilent 5890N GC system, coupled to a 5973 inert MSD using a DB-5MS column (J&W Scientific, 30m x 0.32mm ID x 0.25 μm film; temperature program: 70-5, 5/min-320-5). Identification of compounds occurred using the NIST library (NIST Standard Reference Database; [https://www.nist.gov/srd/nist-standard-reference-database-1a-v14](https://www.nist.gov/srd/nist-standard-reference-database-1a-v14)) in combination with co-elution of standards. Peak area determination was performed using QuanLynx, a quantification software within the MassLynx software (Waters). The experiment was repeated five times.

**Photosynthesis**
Plants grown in separate pots, each containing 25 g of soil, were cultivated in a growth chamber using short-day conditions (11 h light/13 h dark) at 22°C and 70% air humidity. Plants were well-watered for 3 weeks and randomized twice every week. After that, plants were left to dry for the remainder of the experiment or kept well-watered as control. During the drought period, plants were randomized three times a week. To analyze PSII activity in photosynthesis, we measured the Fv/Fm (Fv=Fm ±Fo) value (Fo and Fm are the minimum and maximum values of chlorophyll fluorescence of dark-adapted leaves, respectively), a reliable marker of photo-inhibition (Motohashi & Myouga, 2015). Chlorophyll
fluorescence was measured in leaves at room temperature using a photosynthesis yield analyzer (MINI-PAM, Walz, Effeltrich, Germany). Measurements were taken an hour before the main lights were turned on, with only green light present during the measuring to decrease any photo-inhibition due to light. In exp I, 10 plants were used per genotype and treatment, while in exp II, 17 plants were used.

SUPPLEMENTAL REFERENCES


**Supplemental Figure S1.** Lateral root stages in plc5-1 and wild type seedlings. Developmental stages of lateral root primordia from wild type and plc5-1 seedlings. Values are means ± SE of three independent experiments (n>20).
Supplemental Figure S2. Root development in seedlings of plc3plc5-double mutants.
(a, b) Q-PCR analysis of PLC3- and PLC5-expression levels in wild-type and plc3plc5. Relative expression is based on the expression of SAND. Values are means ± SD (n = 3) for one representative experiment.
(c) Seedling morphology of wild-type and plc3plc5. Seeds were germinated on ½MS medium supplemented with 0.5% sucrose for 4 days, then transferred to ½MS plates without sucrose. Photographs were taken 12 days after germination (DAG).
(d, e) Primary root (PR) length and lateral root (LR) number at 12 DAG. Values are means ± SE of three independent experiments (n>20). Asterisk (*) indicates significance at P<0.05 compared to wt, based on Student’s t test.
Supplemental Fig. S3. PLC5 expression in roots.

**Upper panel.** Histochemical analyses of 10 days-old Arabidopsis seedlings, stably expressing pPLC5::GUS, showing GUS expression at root tip (A), segments (B), and lateral root plus tip at two stages (C, D).

**Lower panel.** Cell- and tissue-specific PLC5 expression (E) as predicted by Arabidopsis eFP Browser (Winter et al., 2007) and comparison to PLC3 (F).
Supplemental Figure S4. Phenotypic analysis of wild type- and PLC5 OE lines grown on soil or agar plates.

Eight-weeks old wild type- and PLC5 OE plants were grown on soil under long day condition and the whole plants (a) and rosettes without inflorescences (b) were photographed. The inflorescence length was measured (c), and the fresh weight of rosette was determined (d). (e) Seedling morphology of wild type and PLC5-OE lines grown on agar plates. Seeds were germinated on ½ MS with 0.5% sucrose for 4 days, then transferred to ½ MS plates without sucrose. Photographs were taken 10 days after germination (DAG). (f) Primary root (PR) length, lateral root (LR) number and average lateral root (ALR) length at 10 DAG. All experiments were repeated at least three times. Values are means ± SD for one representative experiment (n=36). Asterisk (*) marks that PLC5 OEs value is significantly different from wild-type based on Student’s t-test (P<0.05).
Supplemental Figure S5. *plc*5-1 showed no difference with wild type under drought stress. Phenotype of six-week-old wild-type and *plc*5-1 plants. Four-week old soil-grown plants were exposed to drought stress by water withholding for 2 weeks and then photographed. Results are typical of 3 independent experiments.
Supplemental Figure S6. PPI- and PA levels in wild type and PLC5-OE lines.
Five-days old seedlings were $^{32}$P-labelled overnight, and the next day their lipids extracted, separated by TLC and quantified by phosphoimaging. (a) Autoradiograph of a typical TLC, each lane representing 1/5th of the extract of 3 seedlings. (b) Quantification of $^{32}$P-labeled PIP$_2$, PIP- and PA levels in wild type or PLC5 OE lines. Values are calculated as the percentage of total $^{32}$P-labeled phospholipids, and are represented as means ± SD (n=3). This experiment was repeated twice with similar results.
**Supplemental Figure S7.** Soluble carbohydrates in phloem sap of wild type and *plc5-1*. Phloem sap was isolated from 6 weeks-old Arabidopsis plants and their carbohydrates analyzed and quantified by GC-MS. (a) Main carbohydrates, (b), inositol-related compounds. Values are the means of triplicates ± SD from 3 independent experiments.
Supplemental Figure S8. Sugar content in seedlings of wild-type and PLC5-OE lines. Soluble carbohydrates were extracted from 10-day old Arabidopsis seedlings, including shoot (a) and root (b), analyzed by anion-exchange chromatography, and quantified by pulsed-amperometric detection. Mannitol was used as an internal standard. Values are the means ± SD (n=3) for one representative experiment. The experiment was repeated twice with similar results.
Supplemental Figure S9. PSII activity in photosynthesis. Fluorescence measurements of wild type and PLC5-OE2 lines, Fv/Fm (Fv=Fm ± Fo), were obtained one hour before the light period started. Two independent experiments were performed (a, b). For experiment (a), 10 plants were used per genotype, per treatment, and was measured at day 17 after drought initiation, while for experiment (b), 17 plants were used and measured at day 19. Levels were normalized to WT control levels. Asterisk (*) indicates significance at P<0.05 compared to wild type based on Student’s t test.