Supplemental Materials and Methods S1

Plant material and growth conditions

Sorghum (*Sorghum vulgare*) and maize (*Zea mays*) plants were grown under a 12 h photoperiod of 350 μmol photons m^{-2} s^{-1} PAR and a temperature of 28/20°C (light/dark) in soil or hydroponic cultures (García-Mauriño et al., 2003). Arabidopsis Col-0 plants were grown in hydroponics (Araponics, Belgium) under an 11 h photoperiod at 21°C. Leaves of 4-week-old plants were harvested.

Enzyme extraction and PEPC activity analysis

Protein extracts were made as described previously (Monreal et al., 2007) and were incubated in a thermoblock at 30 ºC in the presence of 100 mM Hepes pH 8.0 and complete protease inhibitors (Roche, Mannheim, Germany) in a final volume of 1 ml with or without the indicated phospholipids. PEPC activity was determined at pH 8.0 as described (Echevarría et al., 1990). Phospholipids of different fatty acyl chain composition (synthetic C16:0, 18:1 or diC18:1 as indicated, and natural PI and PI4P species) were all from Avanti Polar Lipids, USA. Synthetic diacylglycrol (DAG) and triacylglycerol (TAG; 18:1 acyl chains) were from Sigma. Lipids in chloroform stocks were dried, rehydrated in 100 mM Tris-HCl pH 8.0 to a concentration of 1 mM and sonicated prior to adding them to the PEPC activity assay mixture. Water-soluble C8:0 PA was added directly from an aqueous stock; end concentration used was below the predicted critical micelle concentration (CMC) of this lipid (King and Marsh, 1987).

Purification and characterization of sorghum PEPC

The enzyme was purified as described (McNaughton et al., 1989) from dark-adapted green leaves (at the end of the dark period) of light-grown plants. Purified enzyme was mixed with an equal volume of glycerol and stored at -20ºC. The malate test was done as described in (Echevarría et al., 1990). The effect of different concentrations of malic acid on PEPC activity was monitored at pH 7.3. The IC_{50} is the malate concentration that causes 50% inhibition of initial PEPC activity.

PA-binding assays

PA bead assays were performed as described (Testerink et al., 2004; Testerink et al., 2007). 350 μg of total sorghum leaf protein was added to 3 μl of Sepharose beads (containing 2.6 μmol PA/ml). Bound fraction was eluted with Laemmli sample buffer, separated on SDS-

PAGE and subjected to western analysis using an anti-*Brassica napus* PEPC antibody (Moraes and Plaxton, 2000).

Subcellular fractionation of sorghum leaf.

Leaves of 4-week-old sorghum plants were ground in liquid nitrogen and protein extraction buffer was added (50 mM Tris pH 7.5, 300 mM sucrose, 5 mM EDTA, 5 mM EGTA, 2 mM DTT, proteinase inhibitors). Subcellular supernatant fractions were obtained using sequential centrifugation steps, involving 1500g for 5 min (3x), 10.000g for 10 min (3x) and 50.000g for 1-hour (twice) centrifugation at 4°C. After each step, the supernatant was transferred to a clean centrifuge tube. The pellet fractions were resuspended in protein extraction buffer. In addition, the 10.000 and 50.000g pellet were washed by homogenization and finally resuspended in protein extraction buffer. Brij58 treatment was used to obtain inside-out vesicles (Johansson et al., 1995; Hardin et al., 2004). Samples were separated on SDS-PAGE and gels were stained with Brilliant Blue G-Colloidal Concentrate (CBB; Sigma-Aldrich, Germany) or subjected to western blotting. Plasma membrane-localized H^+-ATPase was detected using an anti-AHA2 antibody (Palmgren et al., 1991), cytosolic GAPDH with an anti-GAPC antibody (Wang et al., 1997) and anti- barley UGPase was from Agrisera, Sweden.

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