Figure 1

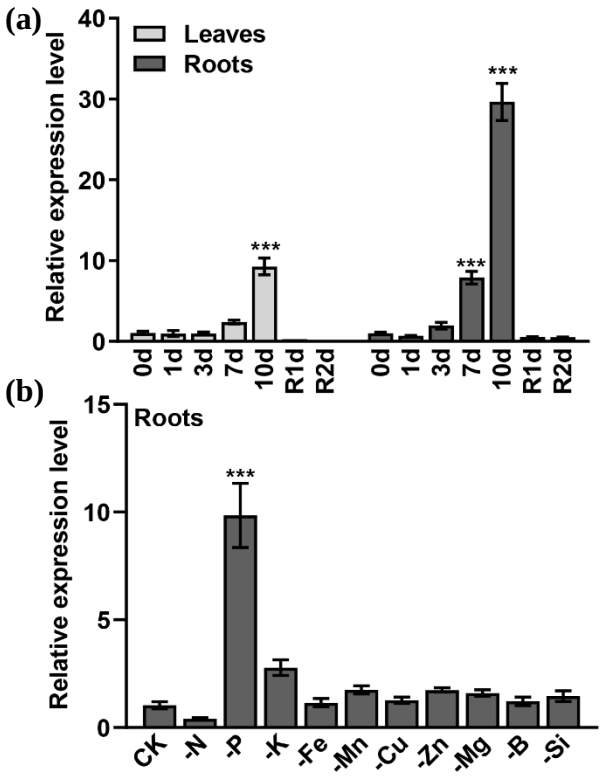

Figure 1. (a) Relative expression analysis of *OsACP1* in leaves and roots during a period of Pi starvation followed by resupply. Germinated seeds were grown in normal nutrient solution for 10 d and then transferred to nutrient solutions without Pi for 10 d, followed by 2 d recovery (R) in normal solution. *OsACP1* expression was normalized to that of *OsACTIN*. **(b) Relative expression of *OsACP1* under different nutrient deficiency conditions.** Ten-day-old seedlings were transferred to normal nutrient solution (CK) or solutions deprived of nitrogen (N), phosphate (P), potassium (K), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), magnesium (Mg), boron (B) or silicon (Si). Roots were sampled after 9d of treatment. Data are means (±SEM) of three replicates. Significant differences compared with the control (0 d or CK) were determined using Student's test (***P<0.001).

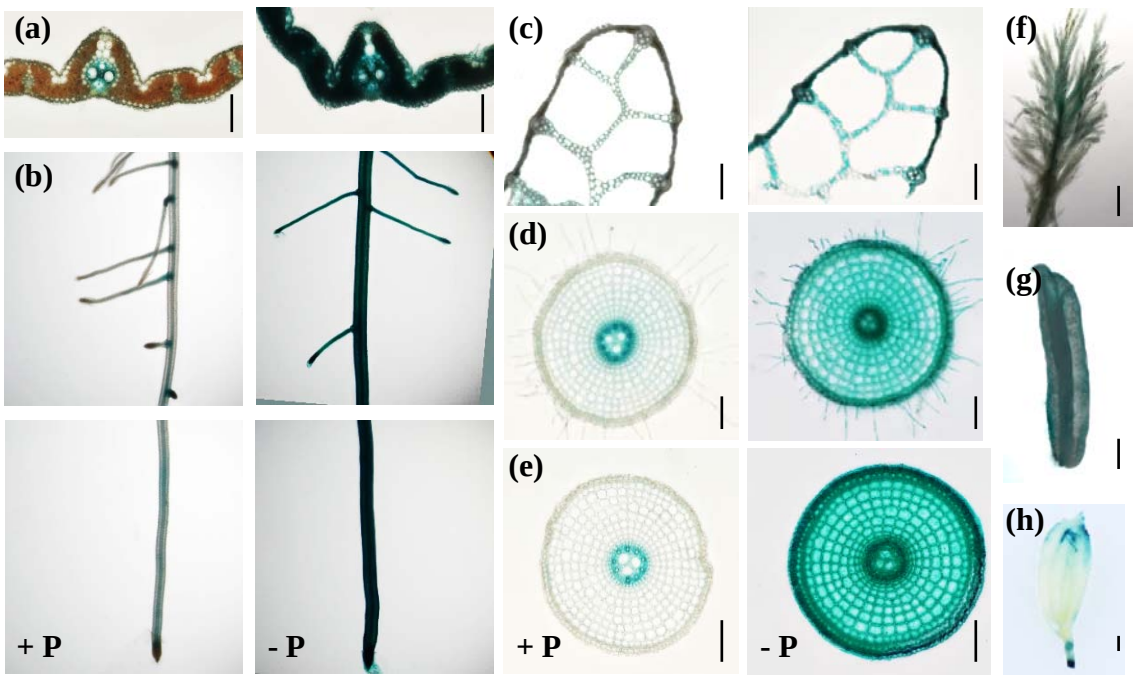

Figure 2. GUS staining of *P_{OsACP1}::GUS* transgenic plants. (a-e) GUS staining of leaves (a), roots (b) and leaf sheaths (c) of *P_{OsACP1}::GUS* transgenic plants under Pi-repleted and Pi-depleted conditions. Transverse sections of leaves (a), leaf sheaths (c), roots of meristem zone (d) and mature zone (e) under Pi-repleted and Pi-depleted conditions. Germinated seeds were grown in normal nutrient solution for 10 d and then transferred to nutrient solutions with (+P) or without (-P) of Pi for 10 d. (f-h) GUS staining of the stigma, anthers and developing seeds of *P_{OsACP1}::GUS* transgenic plants during the blooming stage. Bars = 100 μm.

Figure 3

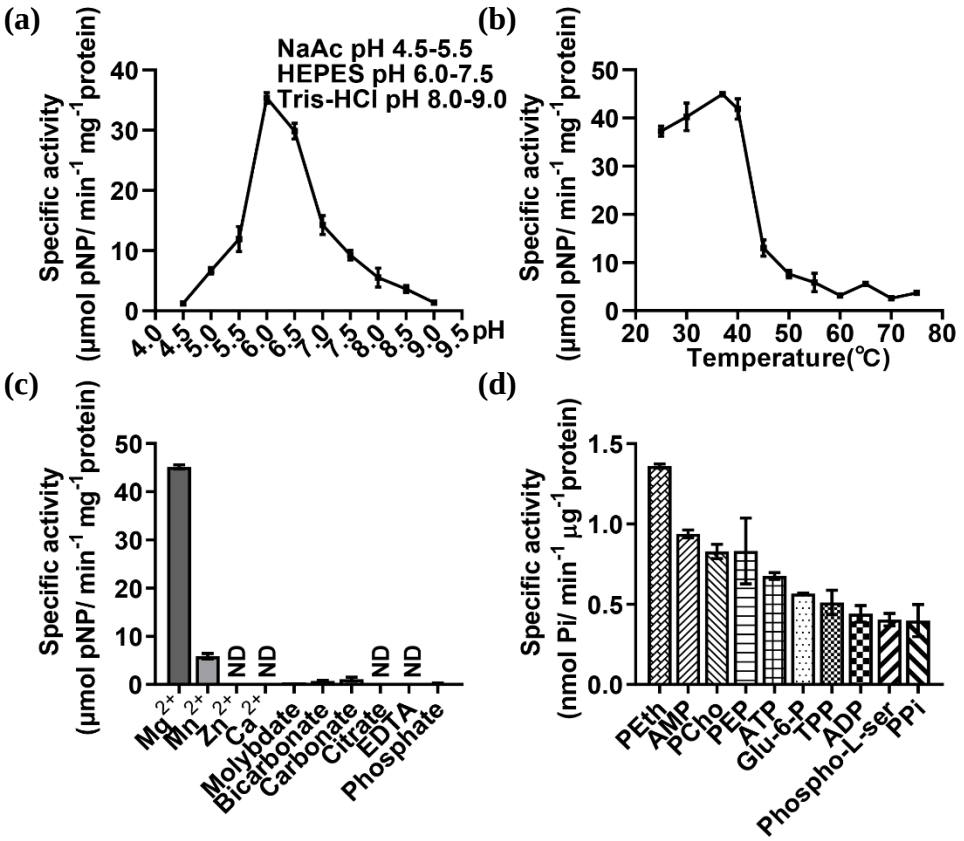

Figure 3. Biochemical characteristics of OsACP1. (a) Effect of pH on APase activity of OsACP1 using pNPP as substrate with different buffers (sodium acetate, HEPES and Tris-HCl). (b) Specific activity of OsACP1 at different temperatures using pNPP as the substrate (pH 6). (c) Influence of cations and anions on OsACP1 activity using pNPP as the substrate (pH 6). ND: not detected. (d) Substrate specificity of recombinant OsACP1. PEth, o-phosphorylethanolamine; Pcho, o-phosphocholine; PEP, phosphoenolpyruvate; Glc-6-P, glucose-6-phosphate; TPP, thiamine pyrophosphate; phospho-L-ser, L-O-phosphoserine; PPi-Na, sodium pyrophosphate.

Figure 4

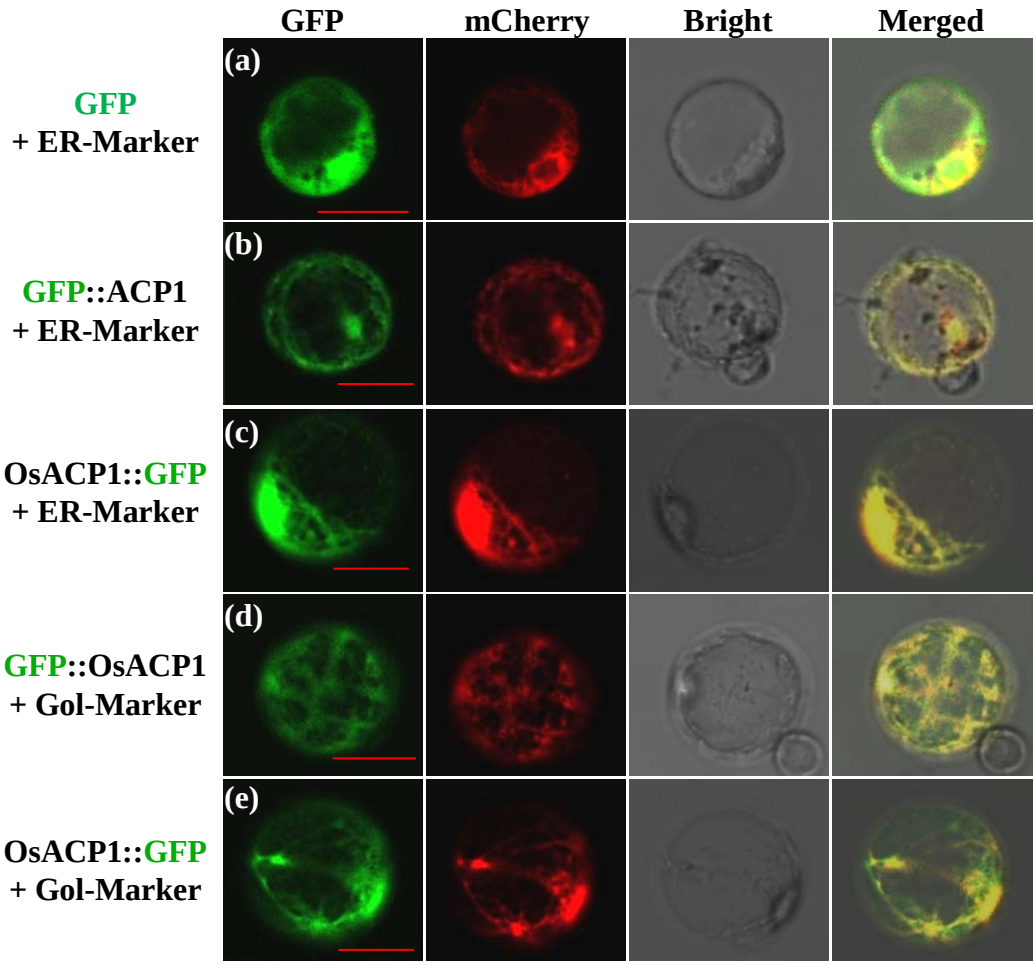

Figure 4. Subcellular localization of OsACP1 in rice protoplasts. N-terminal and C-terminal GFP fusion constructs were transformed with rice protoplasts. The green signals indicate GFP, and the red signals indicate an endoplasmic reticulum marker (CD960::mCherry) or Golgi marker (wave-22R::mCherry). Bars = 20 μm.

Figure 5

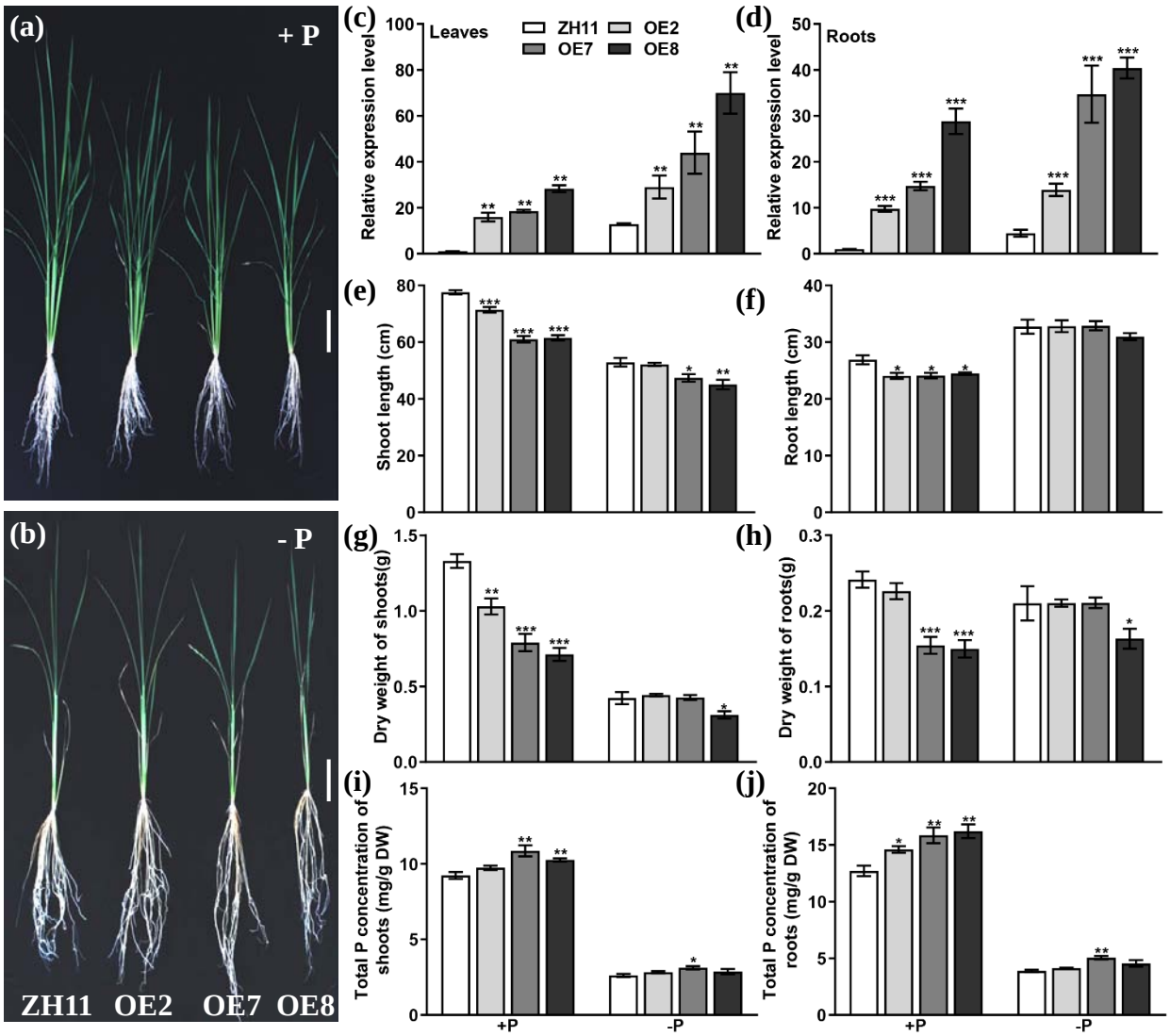

Figure 5. Growth performance, expression levels of *OsACP1*, plant height, biomass and total P concentration of control (*ZH11*) and *OsACP1* overexpression (OE2, OE7, OE8) plants under Pi-sufficient (+P) and Pi-deficient (-P) conditions. (a-b) Growth performance of *ZH11*- and *OsACP1*-overexpressing plants. Bars = 10 cm. (c-d) Relative expression of *OsACP1* in leaves and roots of *ZH11*- and *OsACP1*-overexpressing plants. (e-f) Shoot and root lengths of *ZH11*- and *OsACP1*-overexpressing plants. (g-h) Dry weight of shoots and roots of *ZH11*- and *OsACP1*-overexpressing plants. (i-j) Total P concentration of shoots and roots of *ZH11*- and *OsACP1*-overexpressing plants. Germinated seeds were grown in standard nutrient solution for 14 d and then transferred to +P and –P conditions for 20 d. Data are means (±SEM) of six replicates. Significant differences compared with *ZH11* under the same condition were determined using Student's test (* $P<0.05$, ** $P<0.01$ and *** $P<0.001$).

Figure 6

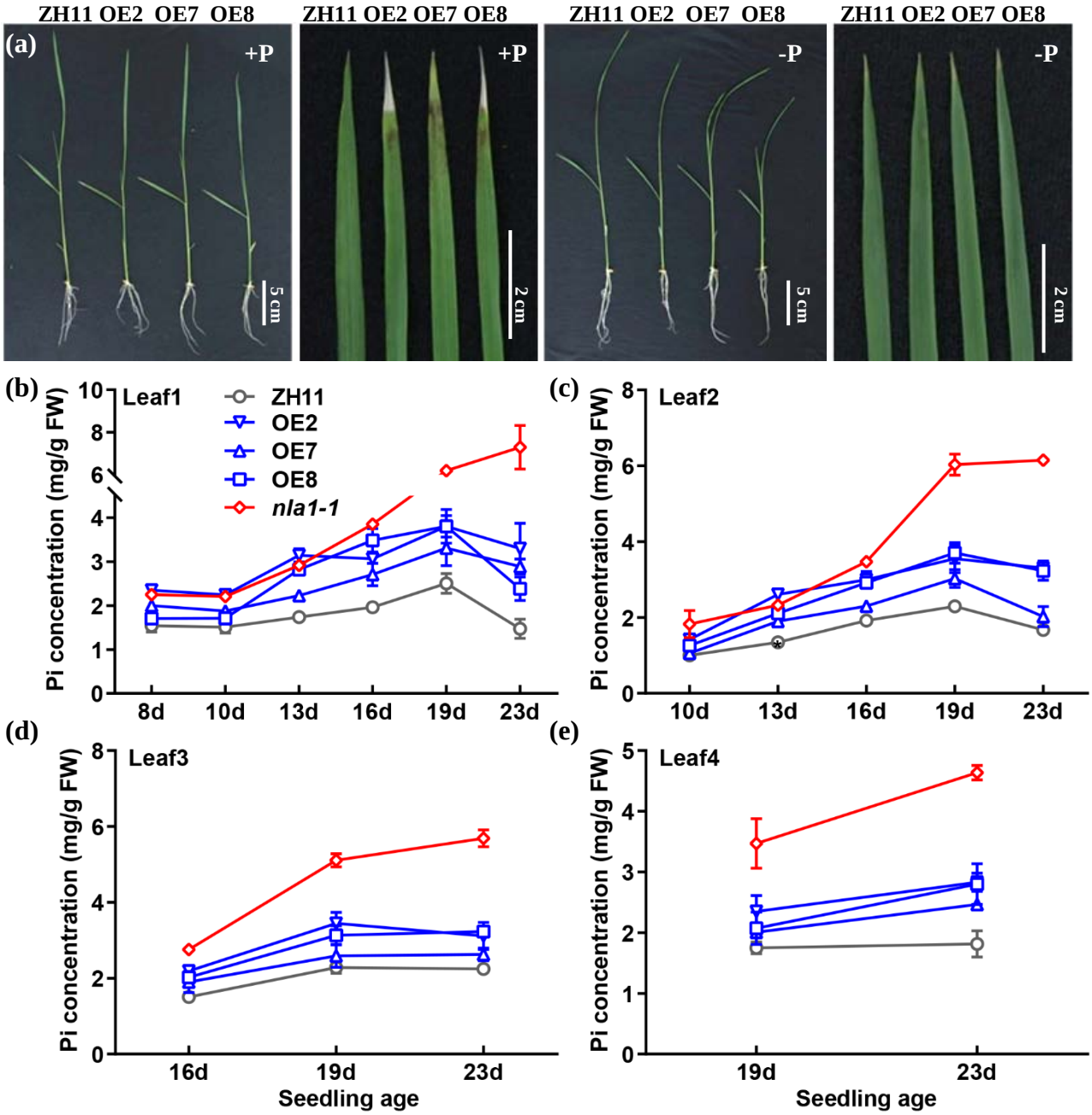

Figure 6. (a) Phenotypes of 10-day-old seedlings of *OsACP1* overexpression lines under Pi-sufficient (+P) and Pi-deficient (-P) conditions. Germinated seeds were grown in nutrient solution with or without Pi for 10 d. **(b-e) Pi concentrations of each leaf over a period of time.** Germinated seeds were grown in normal nutrient solution for 23 days. *ZH11*, control plants; OE2, OE7 and OE8, *OsACP1*overexpression plants; *nla1-1*, *OsNLA1* mutant. Data are means (±SEM) of three replicates.

Figure 7

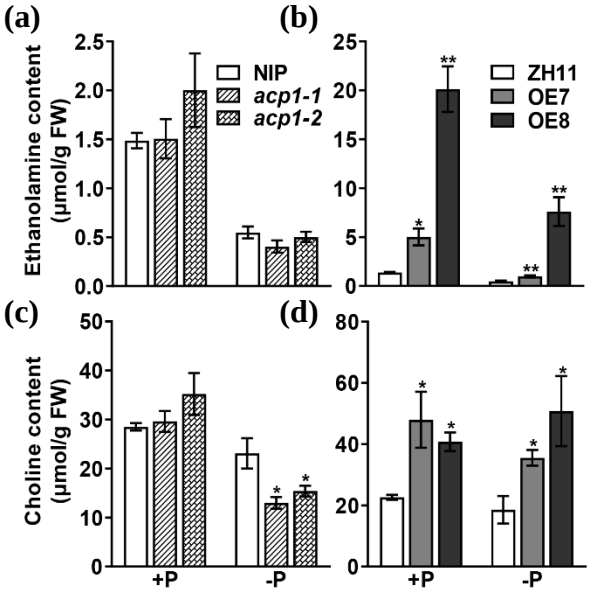

Figure 7. Ethanolamine and choline content in the leaves of *OsACP1* mutants and overexpression plants under Pi-sufficient (+P) and Pi-deficient (-P) conditions. Germinated seeds were grown in standard nutrient solution for 14 d and then transferred to +P and –P conditions for 20 d. Data are means (±SEM) of five replicates. Significant differences compared with the control plants were determined using Student's test (* $P<0.05$, ** $P<0.01$).

Figure 8

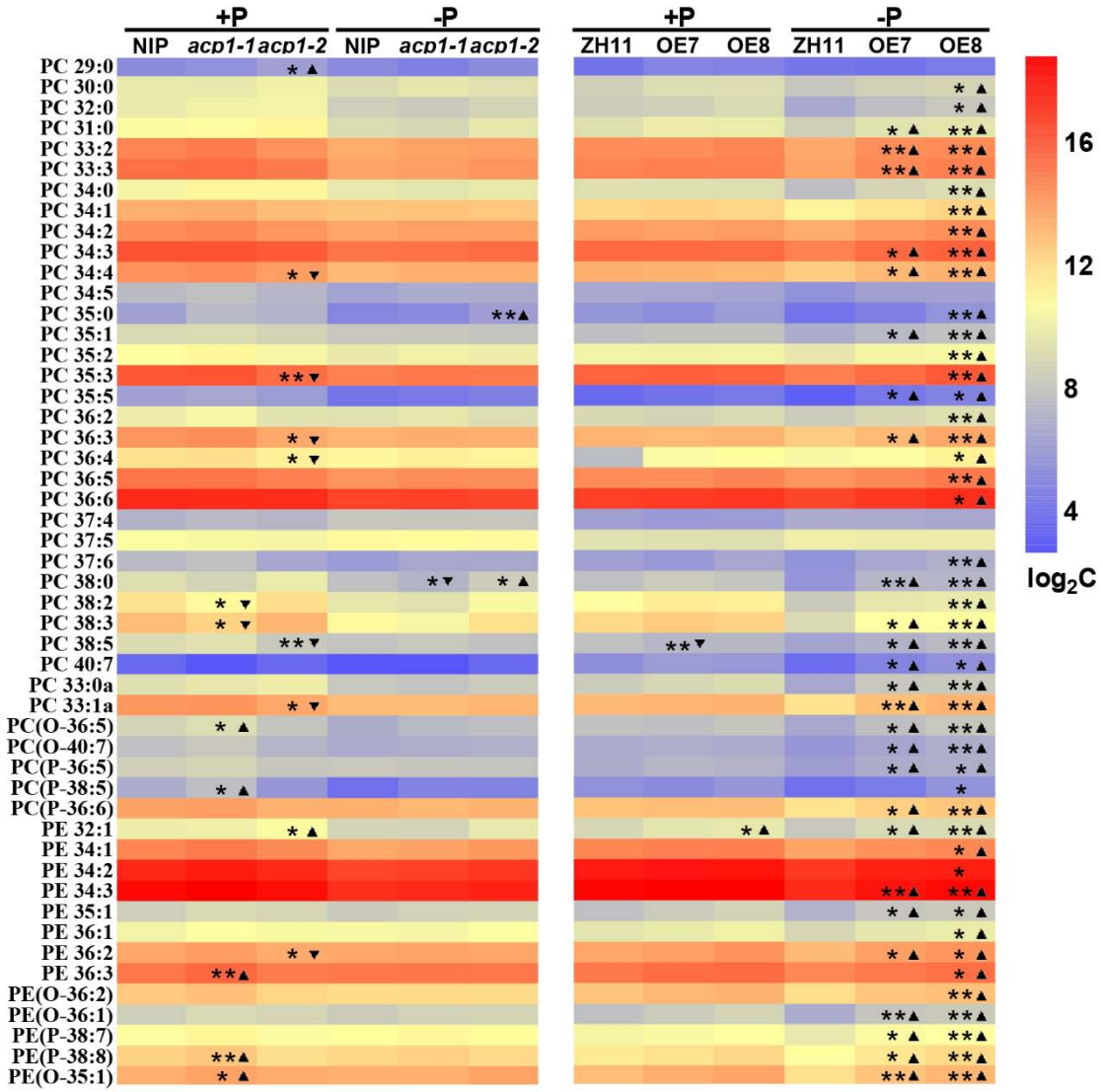

Figure 8. Phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEA) compositions and contents in the leaves of *OsACP1* mutants and overexpression plants under Pi-sufficient and Pi-deficient conditions. Germinated seeds were grown in standard nutrient solution for 14 d and then transferred to +P and –P conditions for 20 d. The content data were normalized by a $\log_2$ calculation method (n=5). "▲" and "▼" indicate that the PtdCho or PtdEA contents were higher or lower than those of the control plants, respectively. Significant differences compared with the control plants under the same conditions were determined using Student's test (* P<0.05, ** P<0.01).

Figure 9

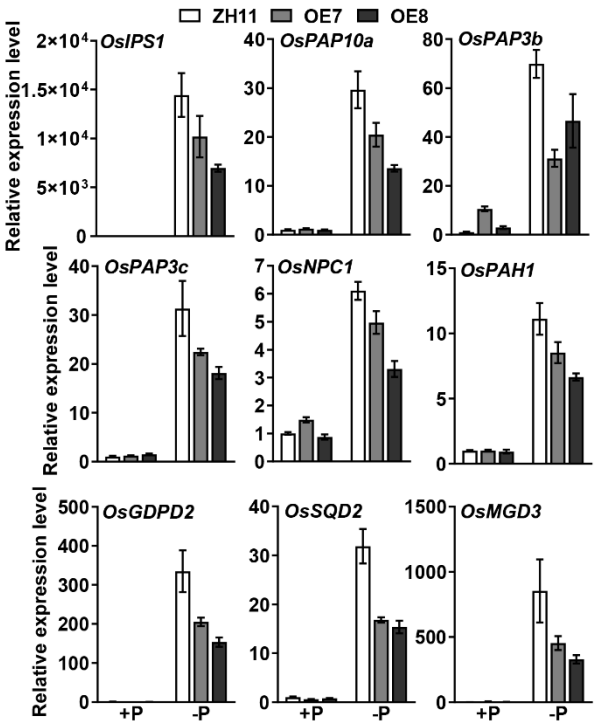

Figure 9. Expression analysis of *PSI* genes in *OsACP1* overexpression plants under Pi-sufficient (+P) and Pi-deficient (-P) conditions. Germinated seeds were grown in normal nutrient solution for 14 d and then transferred to nutrient solutions with or without Pi for 20 d. RNA was extracted from leaves of control (*ZH11*) and *OsACP1* overexpression (Oe7, Oe8) plants. *OsACP1* expression was normalized to that of *OsACTIN*. Data are means (±SEM) of three replicates.