Supplementary Information

Cultivation of plants

Experiment 1: Induction of gradual phosphorus deficiency in hydroponic plants (calibration set)

Barley seedlings were transferred to 32 black 4L containers filled with nutrients dissolved in double deionized water. Nutrient solutions were changed weekly and aerated using steel medical syringes. Each container held ten plants fitted in a lid. In all containers, pH was kept constant at 6.0 ± 0.3 using ultrapure HCl. Plants were cultivated in two growth chambers at a 16/8 h day/night light regime under normal light settings (400 µmol photons m^{-2} s^{-1}) and a constant temperature (20°C). Ten days after transplanting (DAT) the plants were divided into two groups, either maintained under the above-mentioned conditions or exposed to an increased light intensity (750 µmol photons m^{-2} s^{-1}), and a decreased temperature (15°C). The position of all containers was randomized frequently within each climate chamber to avoid any systematic effects. The 16 units in each growth chamber were further divided into four different P treatments including control, P100, P50 and P10. The control nutrient solution contained 200 µM KH_2PO_4, 200 µM K_2SO_4, 300 µM $MgSO_4 \cdot 7\ H_2O$, 100 µM NaCl, 300 µM $Mg(NO_3)_2 \cdot 6\ H_2O$, 900 µM $Ca(NO_3)_2 \cdot 4\ H_2O$, 600 µM KNO_3, 50 µM Fe(III)-EDTA-Na, 2.0 µM H_3BO_3, 0.8 µM $Na_2MoO_4 \cdot 2\ H_2O$, 0.7 µM $ZnCl_2$, 1.0 µM $MnCl_2 \cdot 4\ H_2O$ and 0.8 µM $CuSO_4 \cdot 5\ H_2O$. During the first week after transplanting the concentration of micronutrients was however reduced by 50% in order to avoid EDTA poisoning of the young plants. Initially (<10 DAT), P100, P50 and P10 plants were all supplied with 89 µM KH_2PO_4 to ensure sufficient P supply, while avoiding luxury uptake. In the following period, the KH_2PO_4 concentration was reduced to 45 µM and 9 µM for P50 and P10 treatments, respectively. Twelve days after induction of P50 and P10 treatments, *i.e.* 22 DAT, P100, P50 and P10 treatments were deprived completely of P for the rest of the experimental period.

Experiment 2: Induction of gradual phosphorus deficiency in hydroponic plants (validation set)

The experiment was carried out in a greenhouse under the same climatic conditions as for pre-germination of seeds. 16 hydroponic 4L containers were used, each with four barley plants fitted in the lid. Each container was aerated, the pH kept at 6.0 ± 0.3, and nutrient solution changed weekly similar to experiment 1. The first 10 DAT, all containers were provided control nutrient solution similar to experiment 1. Then four P treatments were induced, control, P100, P50 and P25, each applied to four cultivation units. P100 and P50 treatments were similar to P100 and P50 in experiment 1, whereas P25 treated plants received 22 µM KH_2PO_4. Furthermore, KCl was added to ensure a constant potassium concentration for all treatments, depending on the added amount of KH_2PO_4. 21 DAT, P was completely removed from P100, P50 and P25

treatments. At 28 DAT, P was resupplied by providing all containers with control nutrient solution.

Experiment 3: Induction of phosphorus deficiency in soil cultivated plants (validation set)

Experiment 3 was carried out in a greenhouse under the same climatic conditions as experiment 2. The experimental setup consisted of 30 pots, each holding 4.5 kg soil, divided into 6 different treatments (+P, W3, W4, W5, W6 and –P). The soil was sampled (0-25 cm) from a known P deficient field (sandy loam: Clay 16%, silt 17% and sand 67%, located at University of Copenhagen experimental field station, 55° 40' N, 12° 17' E), air-dried and sieved through an 8 mm sieve. All soil received a basic fertilizer containing N (21.5 %), K (10.6 %), S (3.6 %), Mg (1.0 %) and B (0.02 %) at a rate equivalent to 300 mg N kg^{-1}. The soil for the control treatment (+P) was furthermore fertilized with triple super phosphate (20 % P) at a rate of 0.2 g P kg^{-1} soil prior to plant growth. After pre-germination, nine barley plants where transferred into each pot. Four days later, this was reduced to seven plants. Three weeks after transplanting, W3 treated pots received triple super phosphate in the same amount as for the control treatment, by sprinkling the fertilizer on the soil surface. This procedure was repeated in week 4, 5 and 6 after transplanting (treatment W4, W5, and W6, respectively). The last treatment (-P) did not receive any P fertilizer. The soil was kept moist throughout the experiment by irrigation with Milli-Q water from the top.

Experiment 4: Induction of gradual phosphorus deficiency in hydroponic plants (validation set)

Experiment 4 was carried out in a greenhouse under the same climatic conditions as for experiment 2. 25 hydroponic 4L containers were used, each with four barley plants fitted in the lid. Each container was aerated, the pH kept at 6.0 ± 0.3, and nutrient solution changed weekly similar to experiment 1 and 2. Five containers were provided control nutrient solution, while the remaining 20 containers were provided with P100 solution (89 µM KH_2PO_4). 10 DAT, three treatments were induced from 15 containers initially provided with P100. 3×5 containers each received 45 µM, 22 µM and 9 µM KH_2PO_4 (P50, P25, and P10), respectively. KCl was added correspondingly as in experiment 2. 21 DAT one plant from each cultivation unit was harvested, and P was completely removed from treatments P100, P50, P25 and P10. Other provided nutrients were accordingly reduced to ¾ of the original concentration to account for the harvested plant. At 28 DAT, two plants from each cultivation unit were harvested, and all containers we provided with the control nutrient solution, adjusted accordingly to supply only one plant in each unit.

***Viridis-zb*^{63} – Barley photosystem I mutant**

Seeds from the barley-mutant *Viridis-zb*^{63} were cultivated in vermiculite in a climate chamber with short day settings (8/16 hours day/night light regime under low light settings (150 µmol photons m^{-2} s^{-1}) and a constant temperature at 20°C). After eight days, the plants were moved for darkadaption for 16 hours. The plants did only receive Milli-Q water during the experiment.

Field experiments

Based on low P availability, 16 locations in Denmark were selected for field trials in the summer of 2013 and 2014. At each location, 8 plots of 60 m^2 were placed in a randomized experiment. The entire experiment received a basic fertilizer containing N (21.5 %), K (10.6 %), S (3.6 %), Mg (1.0 %) and B (0.02 %) in amounts corresponding to 110 kg N ha^{-1}. At sowing, 4 plots received triple superphosphate in amounts corresponding to 30 kg P ha^{-1} placed below the seeds, while the remaining 4 plots received no P fertilizer throughout the growing season. All plots were sown with spring barley (*Hordeum vulgare* L. cv. Quench), drilled with 300 seeds m^{-2}, and the YFDL was collected 30 days after sowing. At full maturity, 10–30 m^2 from each plot were harvested and grain yields were recorded. Statistical analysis on grain yields was performed using the Data Analysis add-inn in Microsoft Excel 2010 (Microsoft Corporation, Redmond, Washington, USA).

Historical data

OJIP transients from a range of previously conducted experiments (Hebbern et al., 2009; Husted et al., 2009; Schmidt et al., 2013) were collected to validate the specificity of the observed effect on OJIP transients from P deficient plants. The transients were measured on barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicum* L.) plants, which were cultivated in hydroponics under greenhouse conditions comparable to those in experiment 2 and 3. Different nutrient deficiencies (N, P, K, Ca, S, Mg, Fe, Cu, Zn and B) were induced by removing the single element from the control nutrient solution noted for experiment 1. OJIP transients were collected at a time when nutrient deficiencies were expected to be apparent in the plants.

Chemometric analysis

PCA is an unsupervised method that enables a simple and comprehensive overview of the major variations in a multivariate data set by reducing the number of dimensions with a minor loss of information. Data is presented using the principal components (PC's) as axes, PC 1 explains the most variance in the dataset, and subsequent PC's explain continuously less variance until only noise and/or single sample effects are represented by higher PC's. PCA is described more thoroughly in *e.g.* Martens and Næs, 1989.

PLS analysis is related to PCA. However, in PLS the new sets of principal components are determined to maximize the covariance between measurements (X) and a corresponding set of reference data, Y. The resulting model can be used for prediction of Y-values using new X-data as input. The method is further described in *e.g.* Wold et al., 2001.

Preprocessing

Before PCA and PLS analysis, the OJIP transients were preprocessed. The measured transients displayed unwanted variations both within and between individual experiments. These variations included differences in the maximum fluorescence, the overall slope and offset of the transients due to both the nature of the fluorescence measurements and the spectroscopic hardware used, which adjusts detector gain and offset for each individual measurement. As the observed effect of P deficiency affected the second and higher order components of the OJIP transients, all OJIP

transients were initially normalized by F_0 and subtracted by one to have an origin at zero. The resulting $F(t)/F_0$ transients were then first-order-corrected to further minimize lower order variations within and between experiments. This was achieved by calculating an offset- and multiplicative factor to each individual transient that minimized the difference to the median of the transients from the control plants in the calibration dataset. This effectively adjusted the overall offset and slope of all transients and thus made them more comparable, yet did not affect the second and higher order components of the curves. Mathematically this resembles MSC or SNV preprocessing (Geladi et al., 1985; Helland et al., 1995). All OJIP transients were subsequently differentiated by dividing the change in fluorescence with the difference in time between successive data points. To emphasize the features of the I-P phase of the transient, the differentiated curve were scaled by a composite exponential function designed to enhance the I-step of the OJIP transient and subsequently smoothed using a Savitzky-Golay filter (Savitzky and Golay, 1964), fitting a second order polynomial to a moving window of 15 neighbouring data points.

For the PCA, the full dataset used for the analysis was additionally mean centered to emphasize variations from the mean OJIP transient over the included dataset of transients from both the current and previous experiments (Hebbern et al., 2009; Husted et al., 2009; Schmidt et al., 2013). From experiments 1-4, leaves with a P concentration above 3000 μg g^{-1} DW were marked as healthy control leaves, and leaves with a P concentration below 2000 μg g^{-1} DW were marked as P deficient. Leaves between these boundaries (2000-3000 μg g^{-1} DW) were not included in this particular analysis.

For the PLS analysis, OJIP transients from experiment 1 were used as calibration dataset, and OJIP transients from experiment 2, 3 and 4 were used as validation dataset. Leaves from the same growth unit were pooled in experiment 1, and therefore groups of 5 OJIP transients shared the same reference value. Due to variations between plants in the same growth unit, some of these groups of 5 were poorly represented by one common reference value. For this reason, the root mean squared error (RMSE) for individual transients to the median of the group was calculated, along with the median average deviation (MAD) between the sum of fluorescence intensity around the I-step for the 5 transients in a group. The top 10 percentile of the individual OJIP transients with highest RMSE-values, and the top 15 percentile of the groups of OJIP transients with the highest MAD-values were not included in the PLS analysis as these were not well described by the single reference value associated with them. In experiment 4, the reference value for the plant harvested 21 DAT was used as reference value for all four OJIP transients measured from the four plants in that growth unit. 28 DAT, a specific reference value was measured for two of the remaining three plants; the measurement on the last plant was assigned the mean reference value of the two harvested plants. 31 DAT, a specific reference value was obtained for the OJIP transient from the single remaining plant in each growth unit.

Outlier detection

Data outliers were detected based on interpretations of score plots of the principal components included in the PCA and PLS analyses, as well as plots showing the Q residuals and Hotelling T^2 parameters for each transient. Q residuals represent the residual between the actual transient and

its projection in the principal components of the model; a high Q residual therefor reflects a poor fit in the model. Hotelling T^2 values arises as a generalization of Student's t-distribution (Hotelling, 1931), and a high Hotelling T^2 value reflects a sample that has a high leverage in the model. In these plots, outliers are defined as transients that differ significantly from the majority of the samples; i.e. transients that plot very separate from other transients in score plots, or transients with very high Q residual or Hotelling T^2 values.

159 OJIP transients from 38 independent growth units were included in the calibration dataset. Thus, 161 OJIP transients out of 320 total OJIP transients were not included in the development of the PLS model due to the foliar P concentration of the measured leaves being outside the included 0-3600 µg P g^{-1} DW range, or because they were data outliers. 291 of 382 OJIP transients were included in the validation dataset; the excluded samples were identified as data outliers. For the PCA, 35 OJIP transients were removed from the analysis as outliers, leaving a total of 1228 OJIP transients in the analysis.

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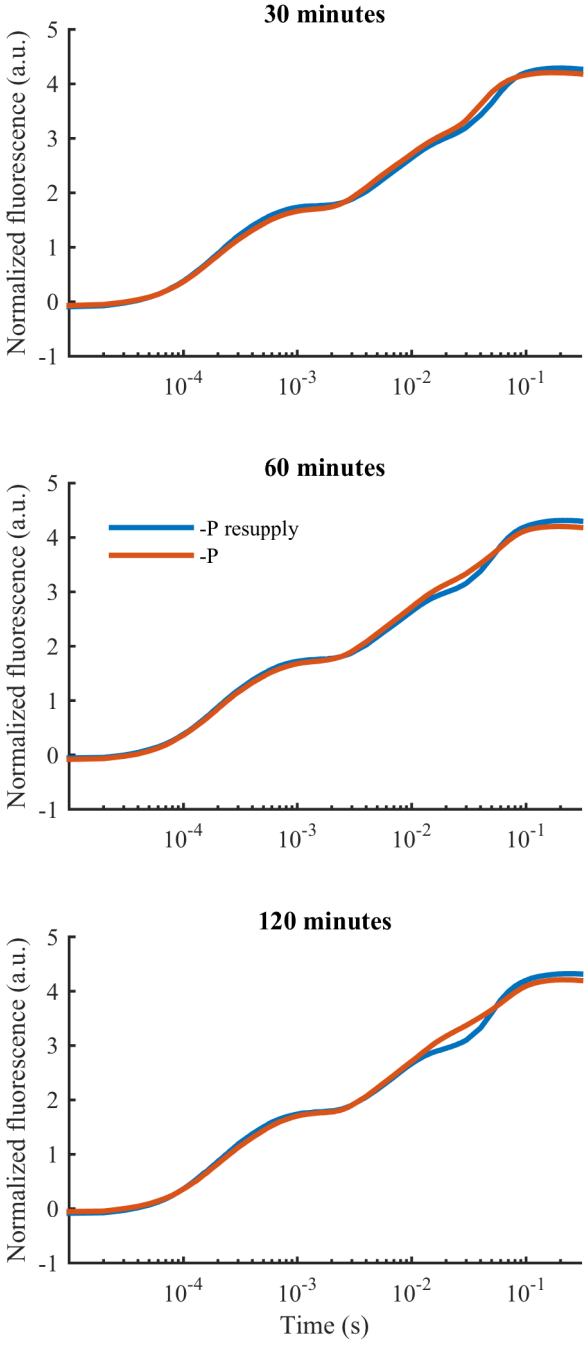


Figure S1: OJIP transient of a P deficient barley leaf resupplied with P (-P resupply), and the OJIP transient of a P deficient barley leaf kept in Milli-Q water (-P) after 30, 60 and 120 minutes. It is seen how the I-step is beginning to re-emerge already after 30 minutes, has grown stronger after 60 minutes and appear fully re-established after 120 minutes.

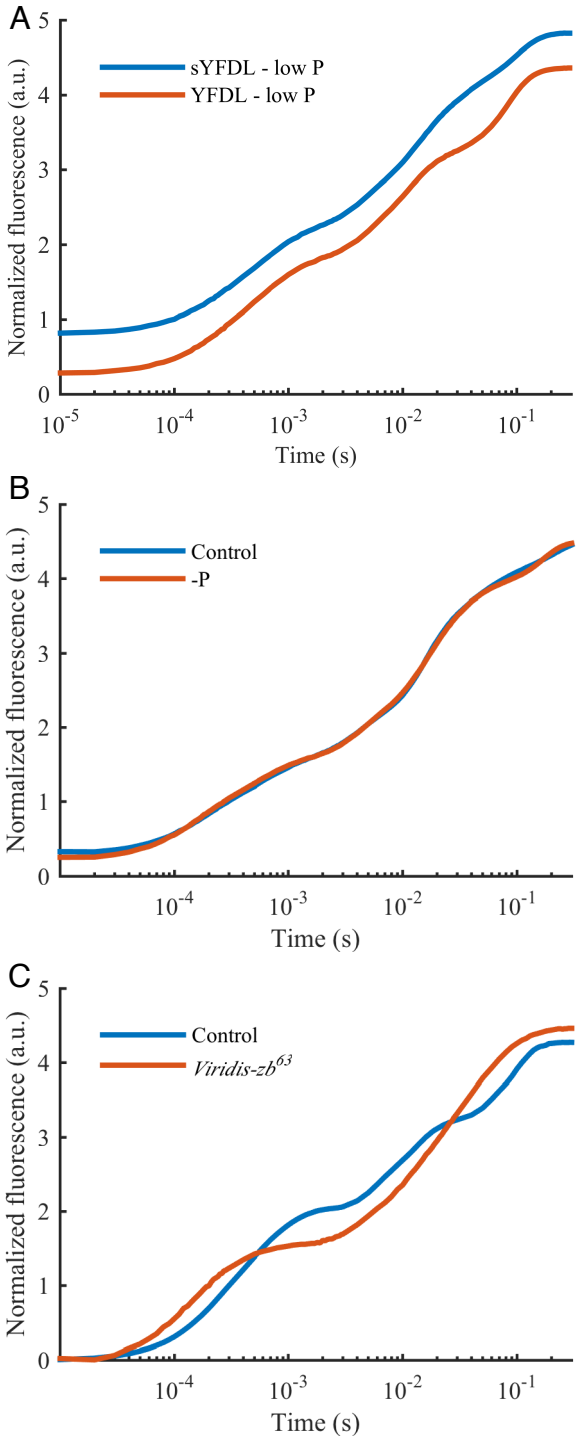


Figure S2: OJIP transients probing aspects of the observed effect of P deficiency ***A)*** OJIP transients from the youngest fully developed leaf (YFDL) and second youngest fully developed leaf (sYFDL) from a barley plant grown under P deficient conditions (YFDL = 1200 µg P g-1 DW) 21 days after transplantation. It is evident that while the I-step is still visible in the moderately P deficient YFDL, it has straightened completely in the sYFDL. The sYFDL transient has been offset by 1 arbitrary unit (a.u) to improve legibility. ***B)*** The OJIP transients from a healthy control and a P deficient barley plant, when illuminated for 60 seconds with far red light to reduce PSI prior to measuring the OJIP transient. The difference observed between the control and P deficient plants under standard measuring conditions has been completely eliminated. ***C)*** OJIP transient measured on a Viridis-zb63 mutant compared to a OJIP transient measured from a healthy control plant in experiment 1. Due to the lack of PSI in Viridis-zb63 the plants were extremely stressed, and hence the maximum fluorescence measured were considerably lower that for healthy control plants. However, pre-processing the transients as shown here allows for a comparison of the shape of the transients despite this difference. It was evident that the I-step was completely absent in the OJIP transients from the Viridis-zb63 mutant.

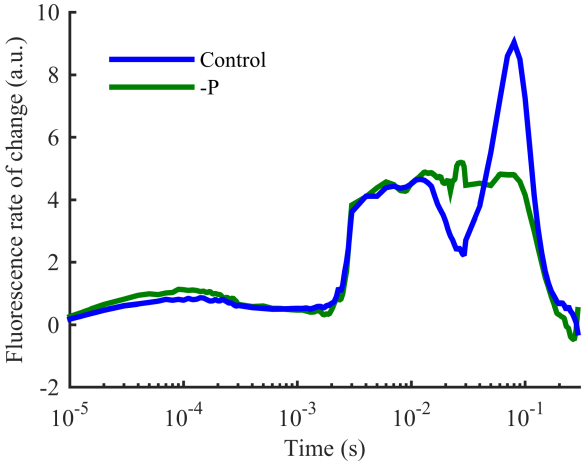


Figure S3: Differentiated, scaled and smoothed OJIP transients from barley. The I-step from a healthy control plant is seen as the clear depression at 0.03 s and subsequent large increase compared to the constant rate of change observed for the OJIP transient from the P deficient plant. All transients were similarly pre-processed prior to multivariate analysis.

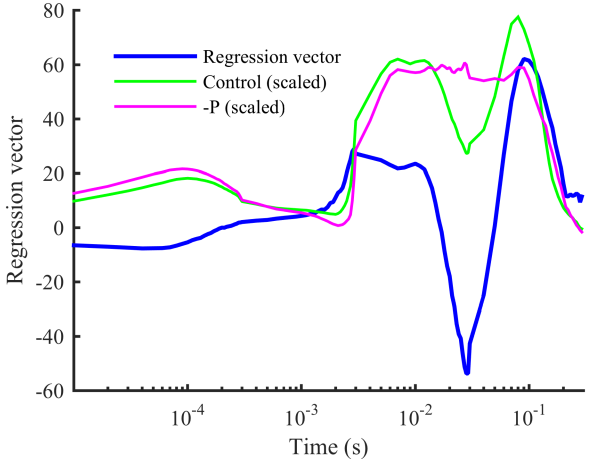
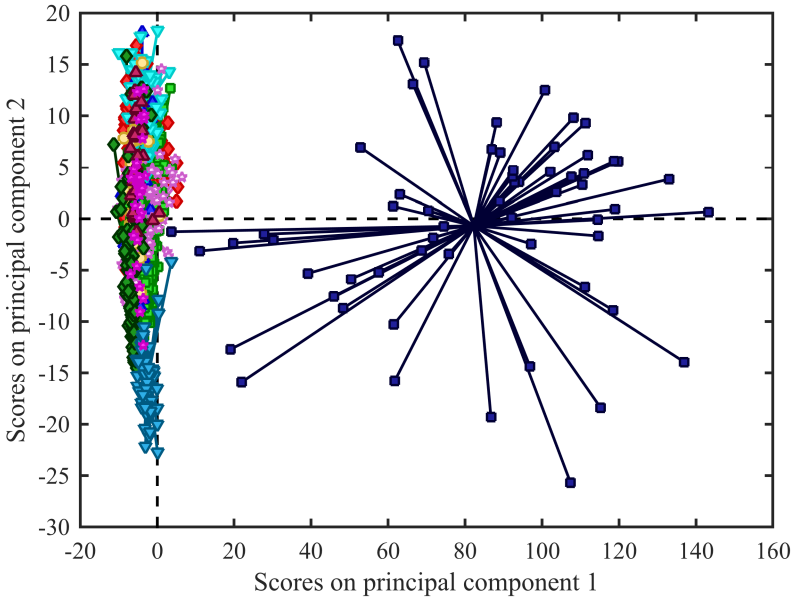
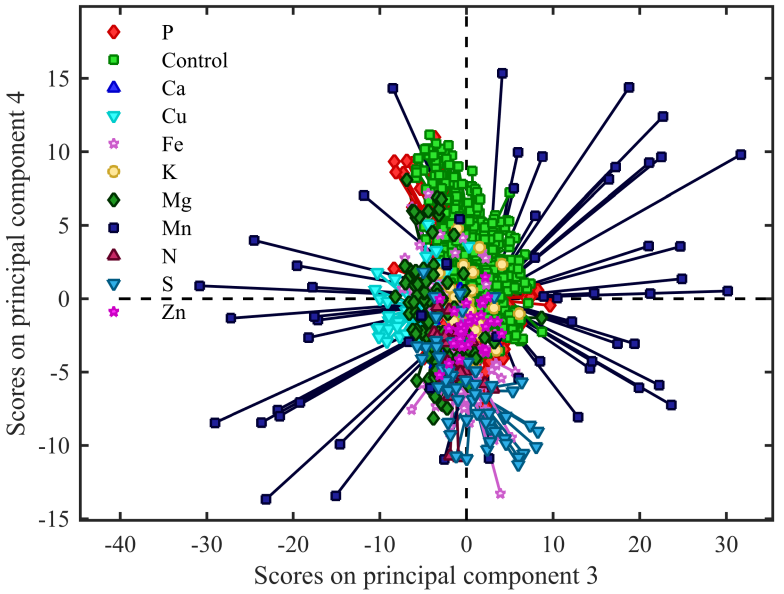


Figure S4: Regression vector for PLS model predicting the P concentration. For comparison, the pre-processed OJIP transients for a control and P deficient barley plant is included (scaled to match the amplitude of the regression vector). It is seen that high PLS predictions correspond to a noted I-step depression (at 0.03 s where the regression vector is negative) and subsequent increase.





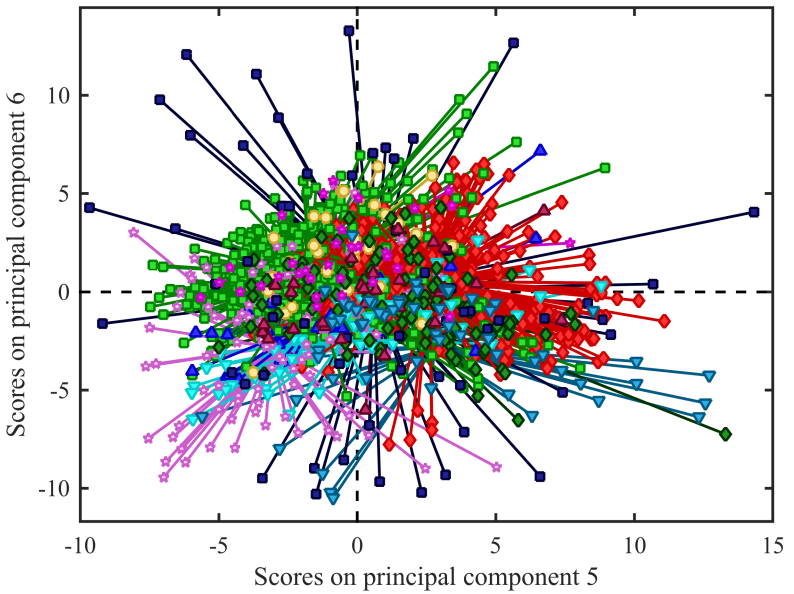


Figure S5: Principal components 1-2 (top), 3-4 (middle) and 5-6 (bottom). PC 1 explains 75.4% of the variance, PC 2: 10.0%, PC3: 4.6%, PC4: 3.2 %, PC5: 2.6 % and PC6: 1.8% of the variance. Mn deficiency is seen to dominate the overall OJIP variability seen in the dataset with clear clustering in PC1 scores, but other PC subspaces show clustering of varying strength of P, S, Cu, Fe and Mg.

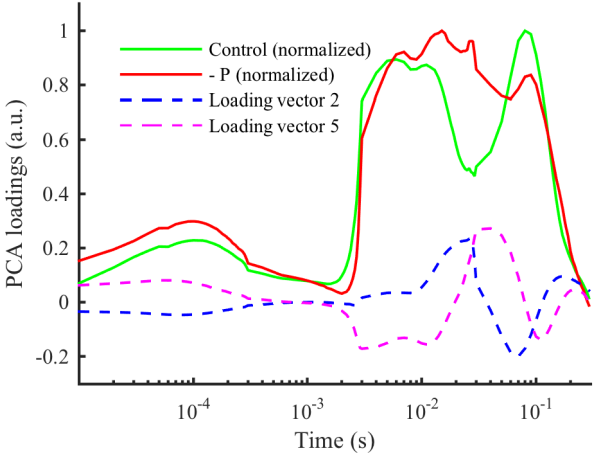


Figure S6: PCA loading vectors 2 and 5 plotted together with pre-processed OJIP transients from a control and P deficient barley plant (scaled to a maximum amplitude of 1 for comparison with the loadings vectors). It is evident from the loading vectors that principal components (PC's) 2 and 5 primarily depend on variations in the shape of the I-step. This corroborates the connection between a change in shape of the OJIP transient around the I-step, and plants suffering from P deficiency as the P deficient plants were seen to cluster distinctly from other deficiencies when plotting the scores of PC's 2 and 5. PC 2 explains 10.0 % of the variation, PC 5 explains 2.6% of the variation.

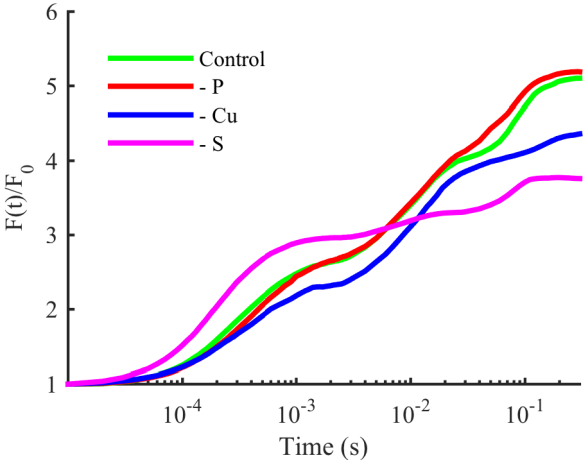


Figure S7: Non-processed OJIP transients (normalized by F0) from a control (green) and a P, Cu and S deficient plant (including both barley and tomato plants). It is evident why both P, Cu and S deficient plants are seen to cluster in a score plot of principal components 2 and 5 as all three appear to affect the shape of the OJIP transient at the I-step, but in different ways. Cu deficiency appear to cause a lower overall increase from the I to P step, whereas S deficient plants appear to have a lower increase between J and I, yet a more pronounced I-step compared to even the control plant. While this strongly suggests that Cu and S deficiency can also be predicted based on OJIP transients, it does not appear to interfere with the effect of P on the shape of the OJIP transient.

Table S1: Elemental concentrations in the YFDL as obtained by ICP-OES. 'A' designates normal temperature and light, 'B' designates low temperature and high light. P1-P3 indicates decreasing levels of supplied P. W3-W6 indicates weeks where pots were resupplied with P, -P did not receive additional P. In experiment 2, plants were resupplied with P at 28 DAT. The results are average mass concentration in dry matter ± standard deviation (n=4 or 5).

				P (%)	Ca (%)	K (%)	Mg (%)	S (%)	Fe (µg/g)	Mn (µg/g)	Zn (µg/g)
Experiment 1 (n=4), hydroponics	21 DAT	A	Control	0.72±0.01	0.95±0.12	7.05±0.28	0.40±0.03	0.57±0.03	90±5	70±15	60±5
			P1	0.37±0.03	1.00±0.04	7.24±0.45	0.41±0.02	0.66±0.01	110±15	100±5	80±5
			P2	0.20±0.01	0.84±0.04	7.85±0.33	0.34±0.02	0.54±0.02	100±10	100±15	85±10
			P3	0.12±0.01	0.49±0.02	8.24±0.12	0.22±0.01	0.42±0.02	80±5	100±5	90±5
		B	Control	0.41±0.04	0.63±0.09	4.24±0.33	0.23±0.02	0.44±0.05	60±5	40±10	30±5
			P1	0.24±0.05	0.43±0.10	5.12±0.73	0.17±0.03	0.45±0.08	70±10	35±10	50±10
			P2	0.13±0.01	0.50±0.08	5.83±0.45	0.19±0.03	0.39±0.05	80±5	50±10	60±5
			P3	0.09±0.01	0.53±0.04	7.71±0.21	0.23±0.03	0.37±0.01	100±60	100±10	105±30
	28 DAT	A	Control	0.58±0.06	0.78±0.18	5.53±0.66	0.35±0.05	0.61±0.05	85±5	45±10	45±10
			P1	0.16±0.01	0.75±0.05	6.36±0.36	0.35±0.02	0.52±0.03	85±5	75±10	50±10
			P2	0.11±0.02	0.61±0.13	7.50±0.20	0.28±0.05	0.44±0.04	85±15	80±20	60±10
			P3	0.06±0.01	0.63±0.05	8.81±0.38	0.30±0.01	0.38±0.02	80±5	130±20	90±5
		B	Control	0.41±0.04	0.65±0.21	4.67±0.25	0.20±0.05	0.43±0.10	70±10	30±10	30±5
			P1	0.11±0.01	0.45±0.11	5.76±0.46	0.16±0.03	0.36±0.05	70±20	35±10	40±10
			P2	0.09±0.02	0.55±0.05	6.80±0.46	0.18±0.02	0.39±0.02	70±10	55±5	55±5
			P3	0.06±0.01	0.52±0.03	7.96±0.37	0.24±0.01	0.38±0.03	80±30	100±5	95±10
Experiment 2 (n=4), hydroponics	21 DAT		Control	0.62±0.04	1.04±0.40	8.36±0.45	0.46±0.16	0.40±0.03	75±5	160±100	105±55
			P1	0.54±0.08	1.17±0.33	8.09±0.57	0.51±0.12	0.43±0.02	80±10	215±85	150±50
			P2	0.21±0.04	1.30±0.05	7.54±0.40	0.58±0.01	0.44±0.01	75±10	250±25	150±10
			P3	0.12±0.07	0.95±0.24	6.98±1.62	0.44±0.11	0.39±0.07	60±15	190±60	125±40
	23 DAT		Control	0.70±0.11	0.54±0.23	8.17±0.32	0.28±0.07	0.45±0.02	70±5	65±25	75±10
			P1	0.44±0.06	0.35±0.02	7.77±0.18	0.21±0.01	0.43±0.02	60±5	50±5	65±5
			P2	0.24±0.05	0.47±0.29	7.20±0.91	0.23±0.10	0.41±0.02	70±10	80±45	80±10
			P3	0.12±0.04	0.70±0.26	7.46±0.24	0.32±0.09	0.38±0.02	70±10	120±30	100±10
	28 DAT		Control	0.65±0.04	0.42±0.08	6.05±0.63	0.26±0.02	0.53±0.04	70±5	30±5	65±25
			P1	0.17±0.04	0.62±0.15	7.11±0.76	0.29±0.05	0.47±0.06	70±10	90±30	70±15
			P2	0.11±0.01	0.43±0.16	6.40±0.20	0.21±0.04	0.37±0.04	70±10	75±30	65±15
			P3	0.09±0.01	0.33±0.08	6.65±0.33	0.18±0.04	0.33±0.02	60±10	75±25	75±10
	30 DAT		Control	0.60±0.11	0.62±0.27	7.10±0.24	0.32±0.07	0.47±0.03	60±5	50±15	65±25
			P1	0.89±0.13	0.64±0.22	7.64±0.52	0.29±0.06	0.47±0.04	60±5	85±35	70±20
			P2	1.03±0.17	0.47±0.12	8.00±0.23	0.23±0.03	0.41±0.02	60±5	70±15	70±10
			P3	1.09±0.20	0.33±0.07	7.73±0.27	0.19±0.03	0.39±0.03	50±10	70±15	80±15
Experiment 3 (n=5), soil	Week 3		Control	0.72±0.05	0.80±0.08	6.78±0.66	0.17±0.01	0.46±0.01	80±5	55±5	45±5
			W3	0.47±0.07	0.85±0.15	7.27±0.93	0.16±0.02	0.47±0.05	90±10	75±15	60±10
			W4	0.48±0.12	1.01±0.22	8.02±1.11	0.17±0.04	0.47±0.04	95±20	80±15	70±10
			W5	0.40±0.09	1.06±0.24	7.10±0.74	0.17±0.03	0.47±0.05	90±15	80±15	50±10
			W6	0.60±0.10	0.92±0.29	7.81±1.46	0.19±0.05	0.53±0.06	105±25	80±15	65±15
			-P	0.51±0.15	0.84±0.15	7.40±0.82	0.17±0.03	0.48±0.06	95±20	70±10	60±20
	Week 4		Control	0.71±0.09	1.04±0.30	6.86±1.06	0.22±0.04	0.54±0.06	100±10	90±20	55±5
			W3	0.47±0.13	1.12±0.34	7.03±0.67	0.19±0.01	0.48±0.04	95±15	120±30	55±10
			W4	0.39±0.05	0.96±0.13	6.69±0.59	0.17±0.02	0.46±0.03	100±10	105±10	60±5
			W5	0.37±0.09	0.98±0.25	6.84±0.65	0.16±0.03	0.45±0.04	100±15	110±30	55±10
			W6	0.35±0.10	1.26±0.16	7.93±0.66	0.20±0.03	0.47±0.03	100±15	135±10	60±10
			-P	0.41±0.08	0.81±0.11	6.68±0.62	0.18±0.02	0.50±0.03	110±15	100±15	65± 10
	Week 5		Control	0.75±0.16	1.75±0.77	4.60±1.18	0.29±0.09	0.58±0.11	110±25	160±70	55±15
			W3	0.83±0.07	1.50±0.80	5.77±0.95	0.26±0.04	0.56±0.05	110±10	165±60	60±5
			W4	0.63±0.26	1.20±0.36	5.43±0.85	0.20±0.03	0.48±0.09	90±20	150±30	55±20
			W5	0.43±0.05	1.02±0.36	6.21±0.81	0.19±0.03	0.53±0.07	90±15	160±35	55±10
			W6	0.56±0.12	0.71±0.14	6.61±1.24	0.17±0.03	0.51±0.09	105±15	125±15	65±15
			-P	0.44±0.08	1.00±0.46	6.11±1.27	0.19±0.05	0.47±0.06	90±30	130±40	60±15
	Week 6		Control	0.50±0.04	0.77±0.19	2.68±0.39	0.15±0.02	0.37±0.04	70±10	70±20	30±5
			W3	0.53±0.22	1.00±0.28	3.62±1.48	0.17±0.03	0.41±0.08	75±15	110±35	35±10
			W4	0.47±0.07	0.84±0.22	3.50±0.33	0.16±0.01	0.40±0.03	70±5	100±15	35±5
			W5	0.63±0.07	1.24±0.29	3.36±0.41	0.17±0.02	0.39±0.05	70±10	170±50	30±5
			W6	0.18±0.08	1.19±0.43	3.58±0.58	0.15±0.02	0.37±0.04	70±5	155±40	30±5
			-P	0.16±0.05	1.14±0.40	3.82±0.52	0.17±0.02	0.39±0.05	75±10	155±50	30±5

Table S2: Harvest data for experiment 1 which was used as calibration dataset. Biomass data are g fresh weight (FW). 'A' designates normal light and temperature conditions; 'B' designates high light and low temperature conditions. P1-P3 indicates treatments with decreasing levels of supplied P. The results are average ± standard deviation (n=12).

			Shoot (g FW)	Root (g FW)	Root/Shoot	Tillers	Chlorophyll (mg/g FW)	Carotenoids (mg/g FW)
21 DAT	A	Control	4.2 ± 0.8	2.2 ± 0.6	0.5	4	2.5 ± 0.1	0.4 ± 0.02
		P1	3.4 ± 0.4	3.0 ± 0.7	0.9	3	2.6 ± 0.1	0.3 ± 0.02
		P2	3.0 ± 0.6	2.7 ± 0.5	0.9	3	2.4 ± 0.2	0.3 ± 0.03
		P3	1.5 ± 0.3	2.4 ± 0.4	1.5	2	2.1 ± 0.2	0.3 ± 0.02
	B	Control	4.1 ± 0.5	3.9 ± 0.8	0.9	5	2.0 ± 0.1	0.3 ± 0.02
		P1	3.6 ± 0.6	3.5 ± 0.9	1.0	4	2.0 ± 0.3	0.3 ± 0.04
		P2	3.0 ± 0.4	3.6 ± 0.8	1.2	3	2.3 ± 0.2	0.4 ± 0.02
		P3	2.1 ± 0.4	3.0 ± 0.6	1.4	3	1.9 ± 0.2	0.4 ± 0.02
28 DAT	A	Control	9.4 ± 1.4	4.2 ± 1.3	0.5	6	2.6 ± 0.2	0.4 ± 0.04
		P1	6.0 ± 0.9	3.7 ± 0.6	0.6	5	2.7 ± 0.2	0.4 ± 0.02
		P2	4.6 ± 1.0	3.4 ± 0.8	0.7	4	2.4 ± 0.2	0.4 ± 0.03
		P3	2.2 ± 0.5	3.0 ± 0.4	1.4	2	2.0 ± 0.2	0.3 ± 0.02
	B	Control	9.8 ± 2.1	6.8 ± 3.4	0.7	7	2.3 ± 0.2	0.4 ± 0.03
		P1	5.9 ± 1.0	5.0 ± 2.1	0.8	6	2.0 ± 0.2	0.4 ± 0.04
		P2	4.4 ± 0.9	5.4 ± 1.2	1.2	5	1.9 ± 0.1	0.4 ± 0.04
		P3	2.3 ± 0.4	3.2 ± 0.6	1.4	3	1.7 ± 0.1	0.3 ± 0.02

Table S3: Results of field trials performed at 16 different locations in Denmark over two consecutive years. Average P concentrations (% in dry matter) at day 30 of the YFDL are shown for plots where no P fertilizer was added (-P), and plots where P fertilizer was added corresponding to 30 kg P ha^{-1} placed below the seeds (+P). Relative grain yield shows the yield of the –P treatment relative to the grain yield from plots that received P fertilizer. A significant (P<0.05) decrease in relative grain yield is observed for the locations highlighted in bold font. For these locations, the P concentration in the YFDL for the –P treatment is ≤0.20%. At location DK 1, no yields loss is observed despite a P concentration <0.20%, this indicates that P is not the limiting nutrient. No consistent reduction in relative yield is observed for leaf P concentrations above 0.23% in the YFDL. (n=4 except for those marked with †, here n=3).

Location	P in YFDL (-P) day 30 (%)	P in YFDL (+P) day 30 (%)	Relative grain yield
DK 1	0.18±0.02	0.24±0.04†	1.00±0.06
DK 2	**0.20±0.01**	**0.24±0.01**	**0.92±0.04**
DK 3	0.32±0.02	0.48±0.02	1.00±0.08
DK 4	0.28±0.10†	0.39±0.05	1.02±0.05
DK 5	**0.16±0.03**	**0.31±0.03**	**0.86±0.11**
DK 6	**0.15±0.07**	**0.26±0.02**	**0.81±0.10**
DK 7	0.33±0.02	0.36±0.02	0.99±0.07
DK 8	0.25±0.01	0.27±0.02	0.95±0.08
DK 9	0.26±0.02	0.34±0.04	0.96±0.07
DK 10	0.30±0.03	0.35±0.02	0.95±0.08
DK 11	0.43±0.06	0.43±0.05	1.01±0.05
DK 12	0.23±0.02	0.28±0.01	0.98±0.02
DK 13	0.26±0.04	0.24±0.01	0.98±0.05
DK 14	0.42±0.04	0.49±0.03	0.93±0.07
DK 15	0.43±0.02	0.45±0.03	1.01±0.04
DK 16	0.42±0.01	0.42±0.12	0.96±0.06

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