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REGULAR ARTICLE

Phosphorus-fertilisation has differential effects on leaf growth and photosynthetic capacity of *Arachis hypogaea* L.

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Abstract

Aims The objectives of this study were to assess how *Arachis hypogaea* L. (peanut or groundnut) responds to different P supplies in terms of growth and photosynthesis, and to determine the optimum P supply and differential P stress thresholds.

Methods We investigated biomass production, leaf expansion, photosynthetic parameters, relative chlorophyll concentration, P700 parameters and chlorophyll fluorescence in a climate-controlled chamber at different P supplies (0.1, 0.5, 1, 1.5, 2 mM).

Results Both deficient and excessive exogenous P supplies significantly reduced leaf growth, relative chlorophyll concentration and dry matter production in two high-yielding peanut cultivars. The optimum P range was 0.8–1.1 mM for peanut seedlings. Through principal component analysis (PCA) and data fitting, we found that the trade-off of the normalised actual quantum yield [Y(II)] and non-regulatory quantum yield [Y(NO)] in photosystem II (PSII) under light is one of the best proxies to determine the suboptimal, supraoptimal, deficient and toxic P supplies, because they are the two key factors with major positive and negative effects of PC1, accounting for 75.5% of the variability. The suboptimal P range was 0.41–0.8 mM and the supraoptimal P range was 1.1–1.72 mM. The suboptimal P supplies corresponded with a leaf P concentration range of 4.8–8.1 mg P g^{-1} DW, while the supraoptimal P supplies corresponded with a leaf P concentration range of 9.9–12.2 mg P g^{-1} DW.

Conclusions Both deficient and toxic P levels severely inhibited leaf growth and photosynthesis of peanut, and these unfavourable conditions were associated with significant reduction of biomass and photosynthesis, and photodamage extending beyond PSII. The trade-off of the normalised Y(II) and Y(NO) is a useful benchmark

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to demarcate deficient, suboptimal, supraoptimal and toxic P-fertilisations levels in *A. hypogaea*.

Keywords Peanut · Phosphorus · Suboptimal · Supraoptimal · Photosynthesis

Introduction

Arachis hypogaea L. (peanut or groundnut) is one of the most important oil crops globally, representing a significant source of protein and vegetable oil. It is an essential component of the edible oil market, especially in China (Bertioli et al. 2016; Fan 2014; Liu et al. 2013; Prasad et al. 2000; Wan 2003; Yu 2008). Being a legume crop, peanut has a high requirement for plant-available soil phosphorus (P) and the P concentration of peanut pods ranges from 10 to 20 mg P g^{-1} DW in the P-inefficient or P-efficient varieties (Yu et al. 2016). Phosphorus is an abundant macronutrient in plant tissues, but the low availability of this nutrient in many highly weathered soils, such as krasnozem and alluvial soils, is often limiting for plant growth and development (Hernández and Munné-Bosch 2015; Schachtman et al. 1998). Even in environments in which P levels are high, much of it is in the form of insoluble phosphate complexes of Ca- and Al- or Fe-oxides and hydroxides, which are not readily accessible to plants (Vitousek et al. 2010). It is noteworthy that P deficiency occurs on half of the world's farmland (Kostic et al. 2017; Lynch 2011; Raghothama 2000).

Inorganic P is usually sorbed onto soil particles, resulting in up to 80% of externally applied P as fertiliser being made unavailable for most plants (Chen et al. 2015b; Lambers and Plaxton 2015). Phosphorus fertilisers are obtained almost exclusively from rock phosphate. However, rock phosphate is a finite natural resource, and the known rock phosphate reserves are conservatively estimated to be depleted in the next few hundred years (Gilbert 2009; Johnston et al. 2014; Pang et al. 2018; Van Vuuren et al. 2010). At the same time, there are problems with excessive application of P fertilisers in many countries including China (Chen et al. 2015a). Excessive application of P fertiliser increases the potential risk of P run-off to surface waters and subsurface drainage, while increasing the farmers' costs (Hammond et al. 2009; He et al. 2011, 2016; Hahn et al. 2012; Kaiser et al. 2009). Furthermore, excessive application of P fertiliser may decrease crop yields due to P toxicity and low efficiency of the use of some trace elements, e.g., zinc (Zn) and iron (Fe) (Broadley et al. 2012; Cakmak and Marschner 1987; Ova et al. 2015; Singh et al. 1988). Thus, it is important to understand the responses of *A. hypogaea* to different P supplies for optimising the use of P fertiliser and other P sources in a sustainable manner.

Many studies have demonstrated that leaves show symptoms of P deficiency or toxicity, including growth reduction, accumulation of anthocyanin, loss of light-harvesting pigments and burnt leaf tips (Carstensen et al. 2018a; Römheld 2012). Phosphorus is essential for the production and functioning of the photosynthetic apparatus (Hammond and White 2008). Consequently, even a marginal P deficiency or toxicity may have major impacts on plant development and the photosynthetic machinery. Phosphorus toxicity symptoms may result from the interaction of mesophyll P with zinc and other micronutrients (Broadley et al. 2012; Cakmak and Marschner 1988; Lambers et al. 2002; Ova et al. 2015). However, we know little about the effects of different levels of P deficiency or toxicity on peanut leaf growth and photosynthesis. Phosphorus deficiency typically reduces leaf expansion rate (LER), the main determinant of total leaf area, due to a decrease in cell production rate and final cell length (Assuero et al. 2004; Kavanová et al. 2006a; Kirschbaum et al. 1992; Rodríguez et al. 1998). This decrease in leaf area, in turn, reduces the production of plant dry matter (Kavanová et al. 2006b). At the physiological level, low P levels affect the ability of plants to utilise sunlight during photosynthesis which may lead to photosystem damage. The functional status of the photosynthetic apparatus can be assessed by various sensitive indicators such as fluorescence and the P700 parameters; P700 - a reaction centre complex, which contains many antenna chlorophyll a molecules and two molecules of a special chlorophyll a with an absorption maxima at 700 nm (P700). These photosynthetic parameters can be measured by approaches involving the use of GFS-3000, the Dual-PAM 100 or Handy PEA chlorophyll fluorometer (Carstensen et al. 2018a, b; Huang et al. 2016; Xu 2013). Previous studies revealed that long-term P deficiency or toxicity reduces net photosynthetic rates (Pn) (Brooks et al. 1988) and stomatal conductance (g_s) in different plant species (He et al. 2011; Zribi et al. 2011). Under short- and relatively long-term low-P stress, there is also a reduction in the PSII quantum yield (Fv/fm), electron transfer rate (ETR), coefficient of photochemical quenching (qP) and effective PSII quantum yield

[Y(II)] in *Oryza sativa* and *Camellia oleifera* (He et al. 2011; Veronica et al. 2017; Xu et al. 2007). The study on barley, soybean, tomato and rice demonstrated that P deficiency might decrease the activity of PSII reaction centres, electron transport rate from PSII to photosystem I (PSI), the activity of Rubisco and other Calvin-Benson cycle enzymes, and amounts of ribulose bisphosphate and 3-phosphoglycerate (Carstensen et al. 2018a; Fredeen et al. 1990; Frydenvang et al. 2015; Xu et al. 2007). However, there are no detailed photosynthesis and growth studies on the response to different P supplies and the resultant leaf P concentration in *A. hypogaea*.

On a global scale, P may be considered as the most limiting macronutrient for peanut nutrition (Yu et al. 2016). Thus, it is important to understand the biological responses of peanut to different levels of P supply by optimising the use of P fertiliser and other P sources in a sustainable manner. We aimed to identify the optimum, suboptimal, supraoptimal and extremely unsuitable concentration (deficiency and toxicity) of exogenous P supplies for peanut, as well as to understand the effects of different levels of P supply on leaf development and photochemical activity. The present study included two widely grown high-yielding peanut varieties supplied with different P levels. We assessed the impacts of different P levels on peanut growth parameters such as leaf growth, gas exchange, and chlorophyll fluorescence.

Materials and methods

Plant material and experimental design

Two common high-yielding peanut cultivars in China: Fenghua 1 and Fenghua 2 (abbreviated as FH1 and FH2, respectively) were used in this study. FH1 and FH2 are peanut cultivars with large and small seeds, respectively. Peanut seeds were pre-germinated in a petri dish for one day at 27 °C, then grown in soil for 7 days, before transferring to the soilless culture system for experiments. After 7 days of soil cultivation, peanut seedlings of uniform size were selected and transplanted into a nutrient solution with different P supplies. There were seven uniform seedlings in each of five P levels, giving a total of 35 seedlings for each cultivar and 70 seedlings for both cultivars. The nutrient solution contained: 2.5 mM KNO_3, 1 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 0.5 mM NH_4NO_3, 0.05 mM Fe-EDTA, 46 μM H_3BO_3, 9.6 μM $MnSO_4$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.03 μM $(NH_4)_6Mo_7O_{24}$. Based on preliminary experiment, five P levels in the form of KH_2PO_4 were used, being P1 (0.1 mM), P2 (0.5 mM), P3 (1 mM), P4 (1.5 mM) and P5 (2 mM). All nutrients were prepared in type 1 ultrapure water (Milli-Q Element, Millipore, Burlington, USA). The nutrient solutions were changed every two days and aerated using steel medical syringes, and the pH of the solution was maintained at 6.0 ± 0.3 using ultrapure HCl. The hydroponic experiment was run in a climate chamber (CONVIRON, Winnipeg, Canada), with a light intensity of 600 μmol quanta m^{-2} s^{-1}, a photoperiod of 16 h, a day/night temperature of 30/25 °C, relative humidity of 60 ± 5%, and air CO_2 concentration at 400 ± 5 μmol·mol^{-1}. The level of nitrogen (N) and potassium (K) was balanced by adjusting the amount of KNO_3 and NH_4NO_3 under the different levels of P supply.

Plant sampling and measurements

Leaf gas exchange was measured on the 3rd youngest fully expanded leaves using an open system of gas exchange equipment (GFS-3000, Heinz Walz GmbH, Effeltrich, Germany) at 1, 7, and 14 days after transplanting (DAT). According to our pre-experiments, we found that short-term (1, 7 DAT) P deficiency or toxicity during early vegetative growth reversibly influenced peanut development including photosynthetic activity and assimilates production. A decline in dry matter production could be avoided or recovered if short-term P deficiency or toxicity (1, 7 DAT) was corrected by revising P fertilisation in time. In our system, a duration of 14 DAT and beyond was arbitrarily defined as long-term. During gas exchange measurements, the leaf cuvette temperature was set to 25 °C and relative humidity at 60%. The CO_2 concentration was kept at 400 μmol·mol^{-1}. An LED array provided a PPFD of 600 μmol quanta m^{-2} s^{-1}. Gas exchange include the net photosynthetic rate (Pn), stomatal conductance (g_s), atmospheric CO_2 concentration (C_a), transpiration rate (Tr), intercellular CO_2 concentration (C_i), leaf stomatal limitation ($L_S = 1 - C_i/C_a$) and water-use efficiency (WUE = Pn/Tr). The relative chlorophyll concentration, fluorescence parameters and P700 parameters were measured at 14 DAT. The software Dual-PAM v1.19 was used to control the Dual-PAM 100 measuring system (Heinz Walz, Effeltrich, Germany) and to measure

chlorophyll fluorescence and P700 parameters on the 3rd youngest fully expanded leaf (ca. 1 cm^2) at 25 °C; all steps were carried out in accordance with the standard protocols of the software (Heinz Walz, Effeltrich, Germany). The fluorescence slow kinetics were measured after a dark adjustment of 30 min. The intensity of saturation pulse light (red light) and actinic light (red light) were set as 10,000 and 132 μmol quanta m^{-2} s^{-1}, respectively. The chlorophyll fluorescence parameters were calculated as follows: The Fo and Fm are the minimum and maximum fluorescence yield of the dark-adjusted sample with all PSII centres open and closed, respectively. Fo' and Fm′ are the minimum and maximum fluorescence yield of the illuminated sample with some PSII centres open and closed, respectively. F is the fluorescence yield measured briefly before applying a saturation pulse. Fv/Fm = (Fm − Fo)/Fm, which indicates the maximal/intrinsic photochemical efficiency of PSII (Kitajima and Butler 1975). Y(II) = (Fm′ − F)/Fm is the actual quantum yield of PSII (Genty et al. 1989). Y(NO) = F/Fm is the non-regulated energy loss in PSII. Y(NO) represents the fraction of energy that is dissipated as heat and fluorescence, and any high value of Y(NO) is a reflection of the inability of the plant to protect itself against damage by excess excitation (Cailly et al. 1996; Klughammer and Schreiber 2008a). Y(NPQ) = 1 − Y(II) − Y(NO) is the regulatory quantum yield in PSII. Y(NPQ) represents the fraction of energy dissipated in the form of heat through the regulated photoprotective NPQ-mechanism (Kramer et al. 2004). ETR(II) = PAR · Y(II) · 0.84 · 0.5 is the relative electron transfer rate in PSII. PAR (μmol quanta m^{-2} s^{-1}) is the photosynthetically active radiation (Genty et al. 1989; Schreiber et al. 1995).

The PSI photosynthetic parameters were measured using a Dual-PAM 100 device based on the P700 signal (the absorption differences between 830 and 875 nm). The quantum yields of PSI were determined using the saturation pulse method (Klughammer and Schreiber 1994). The P700 parameters were calculated as follows: Y(NA) = (Pm − Pm′)/Pm, the quantum yield of PSI non-photochemical energy dissipation due to the acceptor-side limitation. Y(ND) = 1 − P700red is the quantum yield of PSI non-photochemical energy dissipation due to the donor-side limitation (Klughammer and Schreiber 2008b). Y(I) = 1 − Y(NA) − Y(ND) is the actual quantum yield in PSI under light (Klughammer and Schreiber 1994; Klughammer and Schreiber 2008b). ETR(I) = PAR · Y(I) · 0.84 · 0.5 is the relative electron transfer rate in PSI (Klughammer and Schreiber 2008b). Pm is the maximum oxidation state of PSI with the far-red light at 720 nm. Pm′ is the maximum oxidation state of PSI with actinic light. $P700_{red}$ is the P700 reduction parameter under the light.

Three independent peanut seedlings per treatment were sampled at 14 DAT and the plant height, leaf area, leaf mass per unit leaf area (LMA), root to shoot ratio, total plant dry weight, and leaf N and P concentration were measured. Leaf area was measured using a leaf area meter (LI-3000C, LICOR, Lincoln, NE, USA). Leaf relative chlorophyll concentration was estimated with a chlorophyll meter (SPAD-502 Plus, Japan). After samples were oven-dried at 105 °C for 30 min and then at 70 °C to a constant weight, dry weight of root, stem and leaves were recorded separately. The LMA and root to shoot ratio were calculated as: LMA = leaf dry weight/leaf area, root to shoot ratio = root dry weight/shoot dry weight. Dried leaf samples were ground into powder and leaf N and P concentrations were determined using the micro-Kjeldahl and vanadomolybdate methods, respectively (Evans 1983; Westerman 1990).

Statistical analyses

The statistical analyses were performed using 2-way ANOVA in SPSS 19.0. The results were presented as mean values of three independent biological replicates. The P level × variety interaction was examined. All figures present the P level × variety interaction (mean ± SE). If this interaction was significant ($P < 0.05$), then the least significant difference (LSD) at $P = 0.05$ is also presented. Under certain circumstances where there was no significant interaction, but only the P levels had a significant effect ($P < 0.05$), the marginal means for P levels are presented (Table 1). To elucidate the response patterns of FH1 and FH2 under different P levels, a principal component analysis (PCA) was performed using data related to plant growth, leaf nutrient concentrations, gas exchange, chlorophyll fluorescence and P700 parameters. This method reduced the variation inherent of large, multi-dimensional datasets to a few (usually 1–3) most informative axes, called principal components (PCs). PCA is a well-established method to reduce the dimensionality of data and help identify key indicators. In the ordination plots, the PCA preserves the Euclidean distances among samples which implies that closer samples are similar in terms of P supplies, while those that lie on the opposite sides

Table 1 The effects of phosphorus (P) supplies (five levels) on growth and photosynthetic characteristics in two peanut varieties (FH1 and FH2)

	Significance			Marginal means for P levels					
	P level	Variety	Variety× P level	P1	P2	P3	P4	P5	LSD, $P = 0.05$
Plant height (cm)	***	*	ns	18.6	19.9	20.5	19.5	18.5	0.29
Total plant dry weight (g)	***	ns	ns	2.0	2.6	2.8	2.2	1.8	0.06
Leaf area (cm^2)	***	ns	ns	119	132	131	128	117	1.8
LMA ($g \cdot m^{-2}$)	***	ns	ns	65.1	68.1	79.5	67.1	64.2	1.1
Root to shoot ratio	***	ns	ns	0.72	0.66	0.55	0.55	0.55	0.02
Relative chlorophyll concentration (SPAD)	***	ns	ns	26.4	30.4	31.9	30.8	27.8	1.1
Leaf N concentration (mg N g^{-1} DW)	***	ns	ns	25	28	35	31	30	0.8
Leaf P concentration (mg P g^{-1} DW)	***	ns	ns	1.5	5.9	9.0	12.0	12.4	0.4
Tr (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	***	ns	ns	7.4	7.8	7.8	7.8	7.4	0.1
g_s (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	***	ns	ns	233	261	261	259	229	6
Pn (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	***	ns	ns	20.5	22.6	23.4	22.6	20.5	0.4
C_i (μmol·mol^{-1})	**	ns	ns	286	275	275	276	290	4
WUE (μmol $CO_2 \cdot mol^{-1}$ H_2O)	**	ns	ns	2.8	2.9	3.0	2.9	2.8	0.06
Ls	**	ns	ns	0.29	0.31	0.31	0.31	0.28	0.01
Fv/fm	***	ns	ns	0.79	0.81	0.82	0.81	0.79	0.002
Y(II)	***	*	ns	0.44	0.53	0.64	0.55	0.44	0.009
ETR(II)	***	*	ns	24.6	29.3	35.2	30.6	24.3	0.5
Y(NPQ)	***	ns	ns	0.24	0.18	0.12	0.18	0.25	0.007
Y(NO)	***	ns	ns	0.32	0.29	0.24	0.27	0.32	0.006
ETR(I)	***	ns	ns	33.9	36.0	37.4	36.9	32.4	0.5
Y(I)	***	ns	ns	0.61	0.65	0.67	0.67	0.58	0.009
Y(ND)	***	**	ns	0.26	0.24	0.22	0.23	0.30	0.007
Y(NA)	**	***	ns	0.13	0.11	0.10	0.11	0.12	0.006

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

When there is no interaction and there was a significant effect of P levels, the marginal means are presented here

of the axes are most dissimilar to each other. For the PCA, the data were standardized and then computed using the command prcomp () in R (Version 1.1.453). The optimum P supply was determined by fitting the dry weight of FH1 and FH2 with the corresponding P concentration.

Results

Growth responses of peanuts grown under different P supplies

Leaf area increased when the P supply was increased from 0.1 mM in P1 to 1 mM in P3 for both varieties ($P < 0.001$), then decreased when the P supply was further increased in P4 and P5 with leaf area in P5 being similar to that in P1 (Fig. 1a). Leaf area in P3 was ca. 10% greater than that in P1 ($P < 0.01$) and P5 ($P < 0.01$) for both varieties. Both FH1 and FH2 had the highest LMA in P3, while no significant difference between other treatments was found (Fig. 1b). For both varieties, root to shoot ratio in P1 and P2 was significantly higher than that in P3, P4 and P5 (Fig. 1c). Similar to the trend of leaf area, relative chlorophyll concentration (SPAD readings) were also highest in P3 ($P < 0.01$), which was ~20% and ~15% higher than the values in P1 ($P < 0.01$) and P5 ($P < 0.01$), respectively (Fig. 1d). Plant height was highest in P3, followed by P2 ($P < 0.05$) and P4 ($P < 0.01$), and lowest in P1 and P5 ($P < 0.01$) (Table 1,

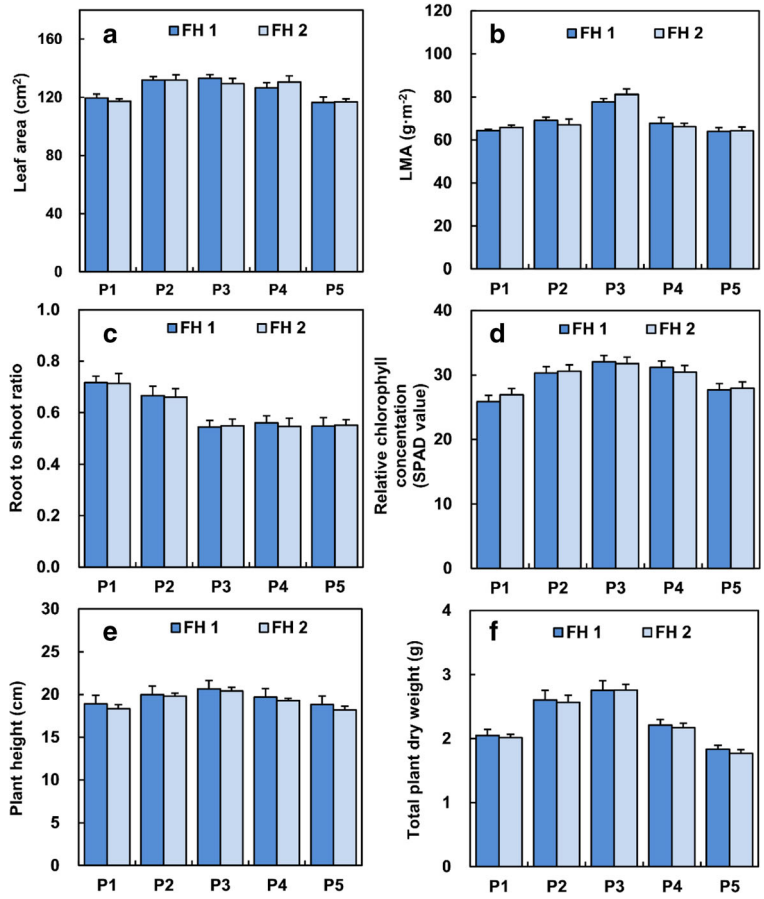

Fig. 1 Response of leaf area (**a**), leaf mass per unit leaf area (LMA) (**b**), root to shoot ratio (**c**), relative chlorophyll concentration (SPAD value) (**d**), plant height (**e**) and total plant dry weight (F) to different phosphorus (P) supplies in peanut plants (mean ± SE, n = 3). See Table 1 for details of the statistical analyses

Fig. 1e). Similarly, total plant dry weight was highest in P3 which was 8%, 27%, 40% and 56% higher than that in P2 ($P < 0.01$), P4 ($P < 0.01$), P1 ($P < 0.01$) and P5 ($P < 0.01$), respectively (Fig. 1f). For leaf area, LMA, root to shoot ratio, relative chlorophyll concentration, plant height and total plant dry weight, we observed no significant effects of peanut varieties or P level × variety interaction ($P > 0.05$, Table 1).

Leaf N concentration increased when P supply was enhanced from 25 mg N g^{-1} DW in P1 to 35 mg N g^{-1} DW in P3 for both varieties, then decreased slightly when P supply further increased in P4 and P5 (31 and 30 mg N g^{-1} DW, respectively) (Table 1, Fig. 2a). For both varieties, leaf P concentration (Fig. 2b) increased when the P supply increased from P1 to P5. Leaf P concentration was lowest in P1, and highest in P4 and P5. No significant difference between the varieties and no P level × variety interaction was found in leaf N and P concentration ($P > 0.05$, Table 1). We fitted the data on total plant dry weight in response to P supplies as a non-linear response curve. Interestingly, we found that the theoretical optimum P concentration was 0.93 mM for favourable peanut seedling growth (Fig. 2c). We would state an optimum P range between 0.8 mM and 1.1 mM when the shoot biomass reached over 97% of the maximum biomass in hydroponics.

The photosynthetic responses of peanuts grown under different P supplies

For all leaf gas exchange parameters, no difference was found among the P levels at 1 and 7 DAT, while a significant difference between P1 and P5 was found from 14 DAT onwards (Fig. 3). There were no significant differences between P2, P3 and P4 at 14 DAT. Photosynthetic rate (Pn), stomatal conductance (g_s), transpiration rate (Tr), and water-use efficiency (WUE) of the two varieties increased from 1 to 14 DAT. Intercellular CO_2 concentration (C_i) of P1 and P5 decreased from 1 to 7 DAT, then increased from 7 to 14 DAT. Intercellular CO_2 (C_i) of P2, P3 and P4 decreased from 1 to 7 DAT, but remained unchanged from 7 to 14 DAT.

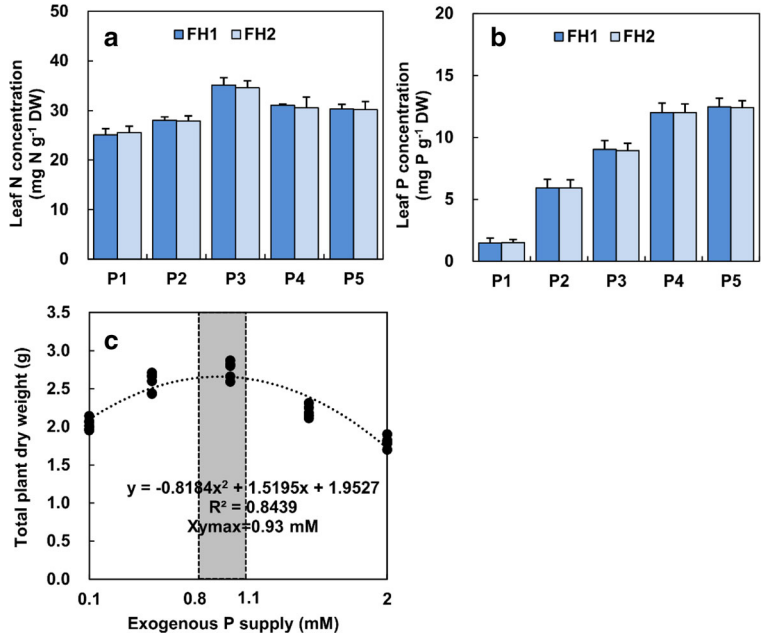

Fig. 2 The responses of leaf nitrogen (N) concentration (**a**) and leaf phosphorus (P) concentration (**b**) to different phosphorus supplies (mean ± SE, $n = 3$). Fitting diagram of dry matter production along different phosphorus (P) supplies in peanut plants (**c**). C, the shaded part is the optimum P range. See Table 1 for details of the statistical analyses

The trend of stomatal limitation (Ls) was opposite to that of C_i. At 14 DAT, all leaf gas exchange parameters including Pn, g_s and Tr showed similar trends, with plants in P2, P3 and P4 showing higher values than that in P1 and P5 (Table 1, Fig. 3). There was no significant difference in these gas exchange parameters between P2, P3 and P4. According to Fig. 3c, C_i depicted an opposite trend to Pn, Tr and g_s, with C_i in P2, P3 and P4 being remarkably lower than that in P1 and P5 for both cultivars. Ls showed a very similar trend to Pn, g_s, and Tr, with plants in P2, P3 and P4 showing higher values than that in P1 and P5 (Table 1, Fig. 3a, b, d and f). No significant difference between the varieties and no P level × variety interaction was found in all gas exchange characteristics at 14 DAT ($P > 0.05$, Table 1, Fig. 3).

Responses of photosystem II (PSII) activity in peanut leaves grown under different P supplies

For both peanut varieties, Fv/fm was significantly lowest in P1 and P5 among all P treatments, and highest in P3, which was significantly higher than that of P1 ($P < 0.01$), P2 ($P < 0.01$), P4 ($P < 0.05$) and P5 ($P < 0.01$), respectively (Table 1, Fig. 4). No significant difference between the varieties and no P level × variety interaction was found in Fv/fm (Table 1).

Leaf Y(II) of plants in P1 and P5 were remarkably lower than those in P2, P3 and P4, with the highest value being in P3 for both varieties (Table 1, Fig. 5a). Both Y(NO) and Y(NPQ) showed a similar trend, but opposite to that of Y(II). Both Y(NO) and Y(NPQ) in P1 and P5 were highest among all P treatments while lowest in P3 (both $P < 0.001$, Table 1, Fig. 5b,c). Relative electron transport rate in photosystem II [ETR(II)] varied significantly among P levels for both varieties ($P < 0.001$, Table 1, Fig. 5d). In both FH1 and FH2, plants in P3 showed the highest ETR(II), which was ~20%, 15% higher than that in P2 ($P < 0.01$) and P4 ($P < 0.01$), while P1 and P5 had similar and lowest values. The lower value in Y(II) in P1, P2, P4 and P5 relative to that in P3 was accompanied with increased values of Y(NO) and Y(NPQ) (Fig. 5e).

Responses of photosystem I (PSI) activity in peanut leaves grown under different P supplies

Significant differences in Y(I), ETR(I), Y(NA), Y(ND) associated with PSI were found among different P treatments (Table 1, Fig. 6). For both varieties, leaves in P3 and P4 showed higher Y(I) and ETR(I) compared with those in P1, P2 and P5, but lower Y(NA) and Y(ND). Both Y(ND) and Y(NA) in P1 and P5 of both cultivars were higher than those in P2, P3 and P4 ($P < 0.01$), which had similar values. Y(ND) in P5 was higher than that in

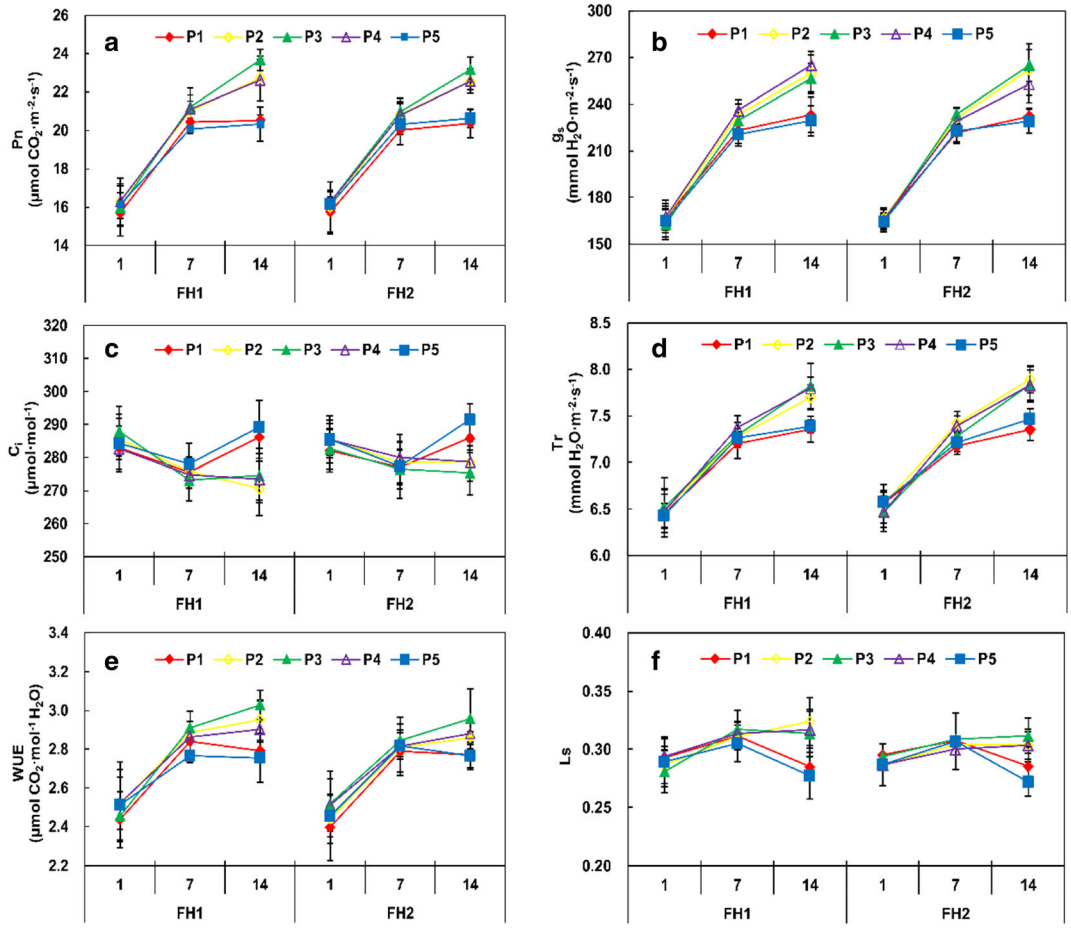

Fig. 3 Responses of leaf gas exchange parameters including photosynthetic rate (Pn) (**a**), stomatal conductance (g_s,) (**b**), intercellular CO_2 concentration (C_i,) (**c**), transpiration rate (Tr) (**d**), water-use efficiency (WUE) (**e**) and stomatal limitation (Ls) (**f**) to different phosphorus (P) supplies at 1, 7 and 14 days after transplanting (DAT) (mean ± SE, n = 3). See Table 1 for details of the statistical analyses of 14 DAT

P1 while Y(NA) in P5 was similar to that in P1 for both varieties (Table 1, Fig. 6b, c). A significant difference in Y(NA) was found between varieties, with Y(NA) in FH2 being 29% higher than that in FH1 taking an average of five P treatments ($P < 0.001$, Table 1, Fig. 6b). The reduction in Y(I) in P1, P2, P4 and P5 relative to that in P3 was accompanied by an increase in Y(NA) and Y(ND) (Fig. 6e). Fig. 6e shows that both varieties exhibited a greater change in the fraction of Y(ND) (ranging from 0.22–0.34) than the fraction of Y(NA) (ranging from 0.09–0.16). No significant difference between the varieties and no P level × variety interaction was found in ETR(I) and Y(I) (Table 1), and no P level × variety interaction was found for Y(NA) and Y(ND) (Table 1).

Establishing the optimum P level and the determination of deficient, suboptimal, supraoptimal and toxic P levels in peanuts

Principal components analysis based on 23 plant traits explained 87.4% of the variance in the first two principal components (Table 2 and Fig. 7). The first component (PC1) represented 75.5% of the variability and accounted primarily for plant height, total plant dry weight, leaf area, LMA, SPAD, leaf N concentration, Tr, g_s, Pn, C_i, WUE, Ls, Fv/fm, Y(II), ETR(II), Y(NPQ), Y(NO), ETR(I), Y(I), Y(ND). The second component (PC2) represented 11.9% of the variance and primarily comprised the root to shoot ratio and leaf P concentration (Table 2). Biplots from PCA analysis clearly showed the

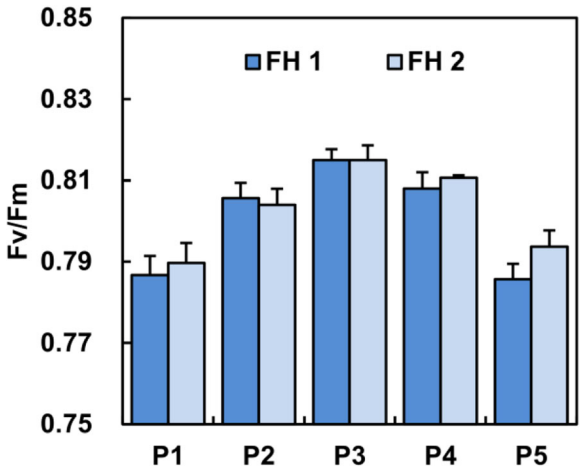

Fig. 4 Response of the maximal/intrinsic photochemical efficiency of PSII (Fv/fm) to different phosphorus (P) supplies in peanut plants (mean ± SE, n = 3). See Table 1 for details of the statistical analyses

differential effects of P (OuP, Optimum P; SuP, Suboptimal and supraoptimal P; EuP, Extremely unsuitable P supply, including deficient and toxic P) levels in the PC1 direction (Fig. 7). The results of PCA indicated that Y(II), representing the actual quantum yield in PSII, and Y(NO), reflecting the fraction of quantum yield that is dissipated as heat and fluorescence in PSII, are two key factors (with major positive and negative effects) in PC1 (Table 2). Therefore, Fig. 8 was prepared using the following determining factors: Y(II) and Y(NO), where the normalised curves are $Y(II)s = [Y(II)' - Y(II)min]/[Y(II)\ max - Y(II)min]$ and $Y(NO)s = [Y(NO)' - Y(NO)min]/[Y(NO)\ max - Y(NO)min]$; the x-axis represented the P supply. According to the fitted curves of Y(II)s [relative Y(II)] and Y(NO)s [relative Y(NO)], when the exogenous P level is <0.41 mM or > 1.72 mM, Y(NO)s value is greater than Y(II)s. The P supply ranging from 0.41–0.8 mM was considered the suboptimal P level, while the P supply ranging from 1.1–1.72 mM was supraoptimal for peanut growth (Fig. 8). Under suboptimal or

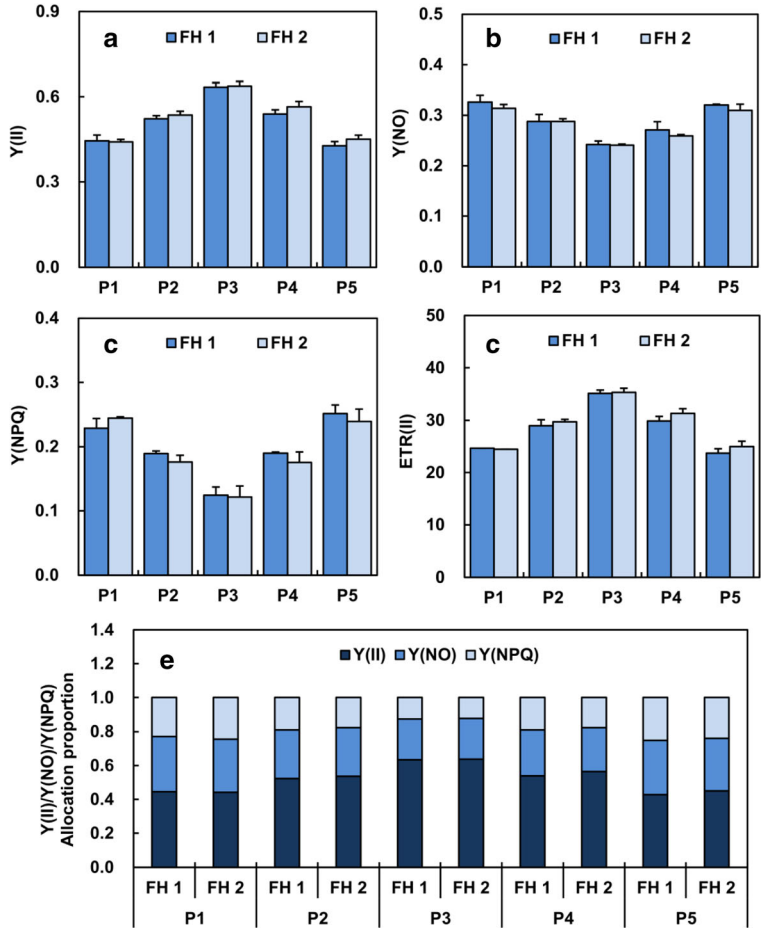

Fig. 5 Responses of leaf Y(II) (**a**), Y(NO) (**b**), Y(NPQ) (**c**), ETR(II) (**d**) and Y(II)/Y(NO)/Y(NPQ) allocation in proportion (**e**) to different phosphorus (P) supplies (mean ± SE, n = 3). See Table 1 for details of the statistical analyses

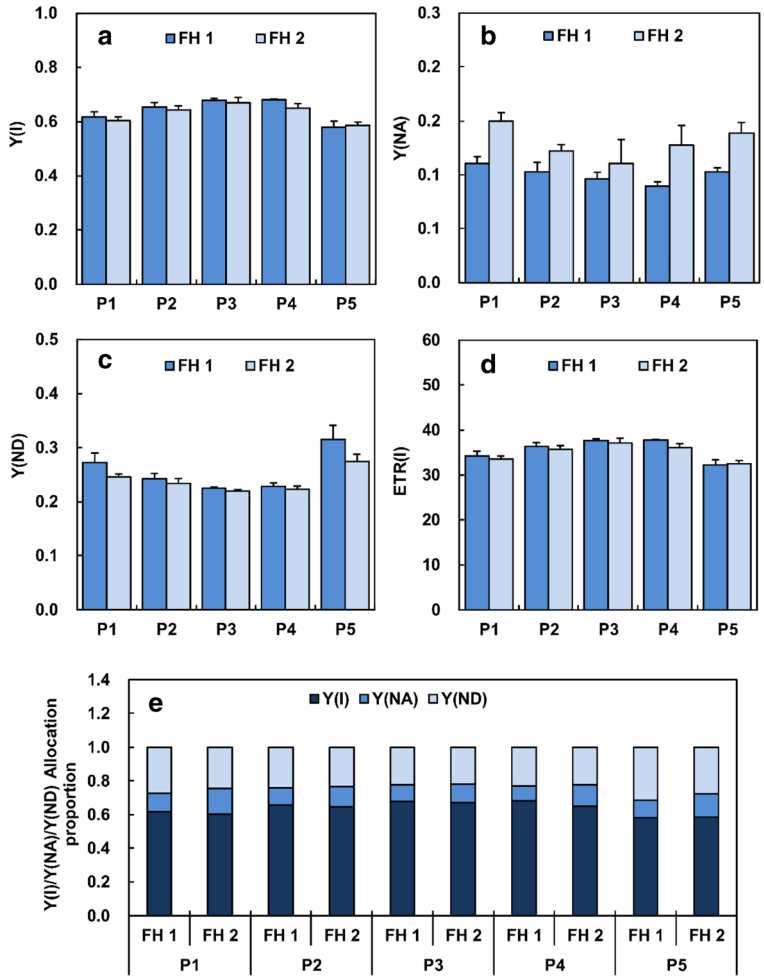

Fig. 6 Responses of leaf Y(I) (**a**), Y(NA) (**b**), Y(ND) (**c**), ETR(I) (**d**) and Y(I)/Y(NA)/Y(ND) allocation in proportion (**e**) to different phosphorus (P) supplies (mean ± SE, n = 3). See Table 1 for details of the statistical analyses

supraoptimal P supply, Y(II)s dominated over Y(NO)s. In addition, it was considered as deficient or toxic P when P supply was either less than 0.41 mM P or greater than 1.72 mM P, as peanut growth and leaf development were severely inhibited with severe photodamages extending beyond PSII.

Figure 9 shows that a non-linear curve was fitted based on exogenous P supplies and leaf P concentrations. We found that the optimum P supply range was from 0.8–1.1 mM, with the corresponding leaf P concentration ranging from 8.1–9.9 mg P g^{-1} DW. Based on this non-linear curve, we conclude that the suboptimal P range corresponded with a leaf P concentration of 4.8–8.1 mg P g^{-1} DW. In addition, the supraoptimal P range corresponded with a leaf P concentration of 9.9–12.2 mg P g^{-1} DW. The deficient and toxic P supply corresponded with a leaf P concentration of less than 4.8 mg P g^{-1} DW and greater than 12.2 mg P g^{-1} DW, respectively.

Discussion

On a global scale, P may be considered as the most deficient element in plant nutrition (Peñuelas et al. 2013; Turner 2008; Vitousek et al. 2010). Peanut is generally grown on P stressed soils in many areas around the world (Yu et al. 2016). There is an urgent need to understand the biological responses of peanut to different levels of P supply by optimising the use of P fertilisers and other P sources in a sustainable manner. Extremely high or low plant-available soil P supplies are associated with poor plant growth and development. In general, an unsuitable P supply reduces leaf growth and photosynthetic CO_2-fixation rates in many plant species (Brooks 1986; Carstensen et al. 2018a; Fredeen et al. 1989; Shane et al. 2003; Weng et al. 2008). The present study demonstrates that different levels of P supplies have

Table 2 Variable loading scores of 23 parameters for peanut varieties FH1 and FH2 exposed to different phosphorus (P) supplies and the proportion of variation of each principal component

	Abbreviations	PC1	PC2
Plant height (cm)	H	**0.94**	−0.08
Total plant dry weight (g)	DW	**0.89**	−0.30
Leaf area (cm^2)	LA	**0.92**	−0.20
LMA ($g \cdot m^{-2}$)	LMA	**0.81**	0.08
Root to shoot ratio	RSR	−0.39	**−0.92**
Relative chlorophyll concentration (SPAD)	SPAD	**0.96**	0.20
Leaf N concentration (mg N g^{-1} DW)	LNC	**0.70**	0.67
Leaf P concentration (mg P g^{-1} DW)	LPC	0.23	**0.91**
Tr (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	Tr	**0.92**	0.05
g_s (mmol $H_2O \cdot m^{-2} \cdot s^{-1}$)	g_s	**0.93**	−0.16
Pn (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$)	Pn	**0.99**	−0.01
C_i (μmol·mol^{-1})	C_i	**−0.90**	0.28
WUE (μmol $CO_2 \cdot mol^{-1}$ H_2O)	WUE	**0.96**	−0.07
Ls	Ls	**0.90**	−0.29
Fv/fm	Fv. Fm	**0.96**	0.08
Y(II)	Y..II,	**0.96**	0.09
ETR(II)	ETR..II.	**0.96**	0.09
Y(NPQ)	Y..NPQ.	**−0.95**	−0.00
Y(NO)	Y..NO.	**−0.95**	−0.22
ETR(I)	ETR..I.	**0.95**	−0.18
Y(I)	Y..I.	**0.95**	−0.18
Y(ND)	Y..ND.	**−0.82**	0.29
Y(NA)	Y..NA.	−0.51	−0.12
Variability (%)		75.5	11.9
Cumulative variability (%)		75.5	87.4

For each parameter, the largest variable loading scores in the two components are in bold

different effects on leaf growth, photosynthetic performance and biomass accumulation in *A. hypogaea*.

Peanut development when grown under different P supplies

Long-term inhibitory effects of extremely low and high P supply on plant growth have been observed in many species such as maize, barley and perennial ryegrass (Assuero et al. 2004; Carstensen et al. 2018a; Kavanová et al. 2006b; Römheld 2012; Shane et al. 2004). However, the responses of P deficiency or toxicity have not been identified for peanut so far. Our study revealed that both deficient and toxic P supply significantly affect leaf growth, relative chlorophyll concentration and dry matter production in two common high-yielding peanut cultivars. In our study, leaf area and LMA were significantly reduced under both deficient and toxic P supply (Fig. 1a, b). It is attributed to the inhibition of leaf expansion rate, maximum relative elemental growth rate (MREGR), relative tissue expansion rate (RTER) and availability of assimilates for leaf growth (Assuero et al. 2004; Kirschbaum et al. 1992; Kavanová et al. 2006b; Rodríguez et al. 1998). In addition, it was suggested that P-fertilisation might influence plant growth through altered cytokinin levels (Horgan and Wareing 1980; Hawkesford et al. 2012; Yong et al. 2014). Consistent with previous studies, the root to shoot ratio of the two peanut varieties under P deficiency was significantly enhanced as more roots were produced in response to the low P supply (Hammond and White 2008; Liao et al. 2001; Yan et al. 2004).

Interestingly, the mild suboptimal and supraoptimal P levels did not induce morphological symptoms in the peanut plants. By fitting the curve between dry matter production and P concentration, the optimum P supply ranges from 0.8–1.1 mM for peanut growth (Fig. 2c). We conclude that the routine half-strength Hoagland's formula targeting peanut seedling should be modified further to increase the P concentrations to 0.8–1.1 mM in order to promote the growth of high-yielding *A. hypogaea* (Hoagland and Arnon 1950; Stanciel et al. 2000). In addition, leaf N concentration increased when P supply was increased from 0.1 mM in P1 to 1 mM in P3 for both varieties, but it decreased when P supply was further increased in P4 and P5 (Fig. 2a). The study by Pang et al. (2011) on 11 herbaceous perennial legume species grown in river sand found that P supply affected shoot N concentration, with most species having higher shoot N concentration when no exogenous P supply was applied than that in all P-supplied treatments. However, an Australian native legume *Kennedia prorepens* reduced its shoot N concentration when exogenous P supply was increased from 0 to 24 μg P g^{-1} soil, but an increase when exogenous P supply was increased further from 24 to 96 μg P g^{-1} soil. The chlorophyll concentration is approximately proportional to leaf nitrogen concentration when P is the limiting nutrient. With increasing nitrogen per unit leaf area, the proportion of total leaf nitrogen in the thylakoids

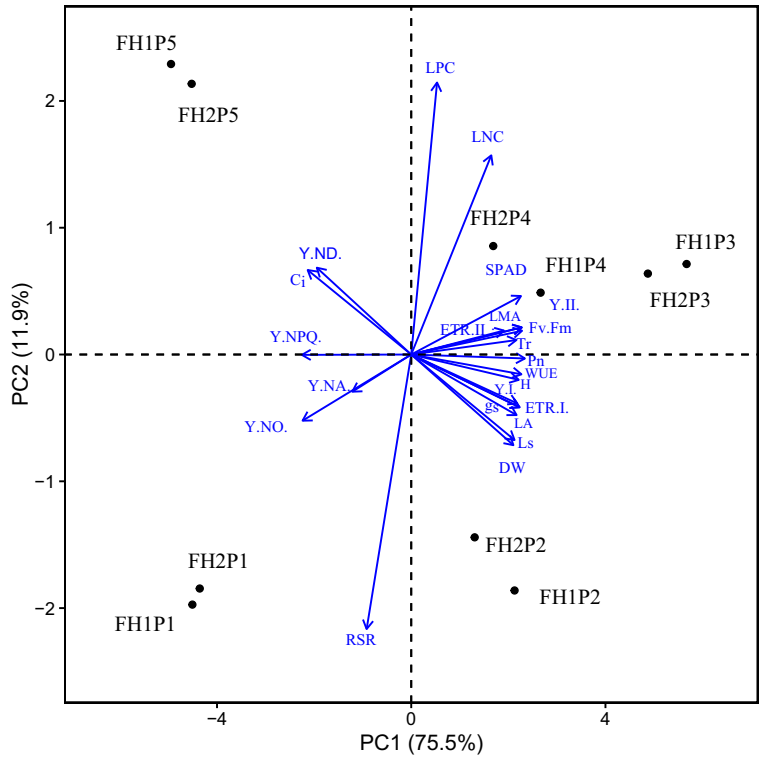

Fig. 7 Principal component analysis (PCA) plots of 23 parameters for the two peanut varieties (FH1 and FH2) growing under different phosphorus (P) supplies including OuP (optimum P), SuP (suboptimal and supraoptimal P) and EuP (extremely unsuitable P including deficient and toxic P). H, plant height; DW, total plant dry weight; LA, leaf area; LMA, leaf mass per unit leaf area; RSR, root to shoot ratio; SPAD, relative chlorophyll concentration (SPAD value); LNC, leaf N concentration; LPC, leaf P concentration; Tr, transpiration rate; g_s, stomatal conductance; Pn, net photosynthetic rate; C_i, intercellular CO_2 concentration; WUE, water-use efficiency; Ls, stomatal limitation; Fv.Fm, Fv/fm; Y.II., Y(II) = Φ_{PSII}; ETR.II., ETR(II); Y.NPQ., Y(NPQ); Y.NO., Y(NO); ETR.I., ETR(I); Y.I., Y(I) = Φ_{PSI}; Y.ND., Y(ND); Y.NA., Y(NA). This is a Biplot with scores + loadings. PC components as Table 2

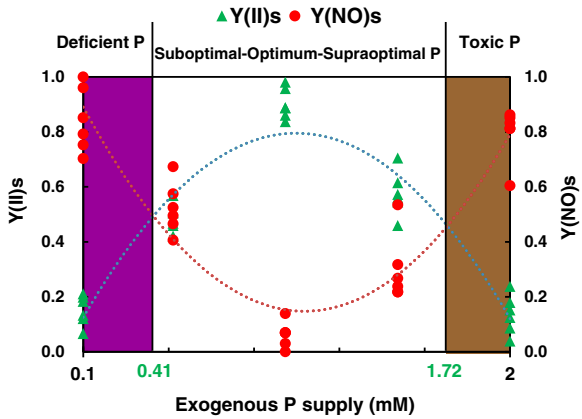

Fig. 8 Fitting diagram of suboptimal and supraoptimal phosphorus (P) supply range in peanut plants. The data of Y(II) and Y(NO) were standardized according to the formula: Y(II)s = [Y(II)′ − Y(II)min]/[Y(II) max − Y(II)min] and Y(NO)s = [Y(NO)′ − Y(NO)min]/[Y(NO) max − Y(NO)min]. Y(II)s and Y(NO)s mean standardized Y(II) and Y(NO) value, respectively; Y(II)min and Y(NO)min mean minimum value in all data of Y(II) and Y(NO), respectively; Y(II)max and Y(NO)max mean maximum value in all data of Y(II) and Y(NO), respectively

remains the same, while the proportion in soluble protein increases (Evans 1983, 1989; Reich et al. 2009). These changes in endogenous leaf N and photosynthesis, whilst receiving similar N nutrition, but different P supplies (Groot et al. 2003; Reich et al. 1997), deserves further in-depth research about

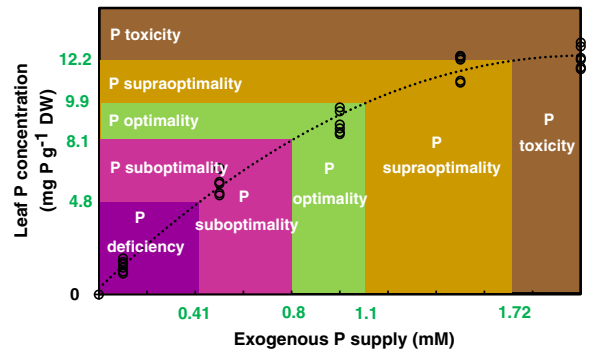

Fig. 9 The fitting diagram of exogenous phosphorus (P) supply and total leaf P concentration for solution culture in peanut plants

a potential form of elements interaction and phosphorus-photosynthesis-nitrogen relation in peanut. For both peanut varieties, leaf P concentration (Fig. 2b) increased when the P supply was higher.

Gas exchange of peanut plants grown with different P supplies

As an essential element in compounds such as adenosine triphosphate (ATP), ribosomal RNA, sugar phosphates, nicotinamide adenine dinucleotide phosphate (NADPH) and phospholipids, there is a large requirement for P in photosynthesis and carbon metabolism (Hammond and White 2008). We observed that P1 and P5 treatments significantly limited carbon-fixation capacity compared with that of P2, P3 and P4 in both peanut varieties from 14 DAT onwards (Fig. 3). This conclusion is also in accordance with previous studies on sugar beet, sunflower and soybean (Fredeen et al. 1990; Plesničar et al. 1994; Terry and Ulrich 1973), where only relatively longer-term treatments under either very low or high P stress decreased photosynthetic rate significantly. Other studies also showed that relatively long-term limiting P supply dramatically reduces photosynthesis due to reduced growth and low sink demand (Pieters et al. 2001). In the present experiment, there was a reverse trend for changes in g_s and C_i in peanut leaves with a change of P supply which is consistent with previous studies (Singh et al. 2013; Xu 1997; Warren 2011; Zhang et al. 2014a, b, c). Based on our results (Fig. 3), photosynthesis in peanut leaves was negatively impacted by non-stomatal limitations, because Ci increased and Ls decreased under deficient and toxic P stresses. Therefore non-stomatal limitations were the dominant factors affecting photosynthesis under deficient and toxic P stresses. The results from the present study are consistent with previous studies on spinach, barley, sugar beet, white lupin and *Eucalyptus* (Campbell and Sage 2006; Foyer and Dietz 1986; Rao and Terry 1989; Thomas et al. 2006).

Effects of leaf photosystems in peanut plants grown under different P supplies

Severe P deficiency or toxicity can lead to alterations in the photosynthetic apparatus and photosystems, thus causing photo-oxidative stress (Hernández and Munné-Bosch 2015). Through further analysis of peanut PSII and PSI fluorescence parameters, we found that leaf Y(II) and Fv/fm under deficient or toxic P supply was remarkably lower than that at suboptimal or supraoptimal P supply, with the highest value being in P3 treatment for both varieties (Table 1, Fig. 4 and 5a). Consistent with the previous findings, maximal photochemistry efficiency and actual quantum yield in PSII under light were reduced significantly in P-deficient or P-toxic plants due to photo-oxidative damage (Hernández and Munné-Bosch 2015; Weng et al. 2008; Zhang et al. 2014a, b, c). Both Y(NO) and Y(NPQ) showed a similar trend, but opposite to that of Y(II). Both Y(NO) and Y(NPQ) of P1 and P5 were highest among all P treatments, and lowest in P3, and medium in P2 and P4 (Fig. 5a–c). Quenching of PSII fluorescence depends on the competition between the photochemical and non-photochemical processes that lead to the relaxation of the excited state of the chlorophyll molecules (Krause and Weis 1991). Phosphorus deficiency and toxicity generally increase the non-photochemical quenching, and decrease the photochemical dissipation of excitation energy from PSII. In addition, as previously reported, P deficiency induces lumen acidification, which activates the regulated photoprotective NPQ-mechanism (Carstensen et al., 2018a, b; Kramer et al. 2004). In addition, our results show that P toxicity also activated the regulated photoprotective NPQ-mechanism. In parallel, Y(NO) reflects the fraction of energy that is dissipated as heat and fluorescence (Cailly et al. 1996; Klughammer and Schreiber 2008a), and the high values of Y(NO) in P1 and P5 indicate the inability of the plants to protect themselves against photodamage in PSII. Therefore, both P deficiency and toxicity caused the significant reduction of the leaf gas exchange and plant growth.

Generally, PSII is accepted to be the most vulnerable part of the photosynthetic apparatus to photodamage under stresses. The excess energy causes damage to PSII, leading to the sustained decline of its efficiency (Havaux and Davaud 1994; Melis 1999). The damage and repair of PSII reaction centres was almost simultaneous under general nutrient stress (Xu 2013). However, some other studies have confirmed that the preferential PSI photoinhibition may also occur under low-light stress in tomato and tobacco (Hernández and Munné-Bosch 2015; Li et al. 2004; Meng et al. 2017). The conversion of excitation energy into the energy of separated

charges and water-plastoquinone oxidoreductase activity is inadvertently coupled with the formation of reactive oxygen species (ROS). Excess ROS induces peroxidation of thylakoid membrane lipids, degradation of the D1 protein, and photoinhibition of PSII and PSI (Carstensen et al. 2018a; Zhang et al. 2014a, b, c; Zivcak et al. 2015). In our study, the photodamage under P deficiency and toxicity stresses seems to be spreading from PSII to other parts of the photosynthetic electron transport chain with increased Y(NO) (Frydenvang et al. 2015; Krause and Weis 1991). In addition, we also found that both Y(ND) and Y(NA) in P1 and P5 of both peanut cultivars were higher than those in P2, P3 and P4. Indeed, both peanut varieties showed a more significant change in the fraction of Y(ND) (ranging from 0.22–0.34) than the fraction of Y(NA) (ranging from 0.09–0.16) (Fig. 6b, c). It indicated that leaf growth and photosynthesis under deficient and toxic P supply including P1 and P5 are severely inhibited with photodamage extending beyond PSII, and, particularly, the donor side inhibition of PSI is more serious compared with that of the acceptor side of PSI under deficient and toxic P supply. Previous research in barley through assessing chlorophyll a fluorescence transients (OJIP transients) showed that severe P deficiency induces the donor side inhibition of PSI which affects electron transport to PSI due to the accumulation of protons in the thylakoids and lumen acidification resulting from the inhibition of ATP synthase activity (Carstensen et al. 2018a). In barley and tomato, the variations during the I-P phase of OJIP transients under P deficiency show an impact on the photosynthetic electron transport chain from PSII to cytochrome *b6f*, thereby reducing the probability of PQH_2 oxidation, ultimately reducing the linear electron flow to PSI (Carstensen et al. 2018a, b; Frydenvang et al. 2015; Joly and Carpentier 2007). Phosphorus deficiency affects both Rubisco activity and the capacity for ribulose bisphosphate regeneration (Brooks et al. 1988). The limitation of Calvin cycle reduced the linear electron flow. Previous studies on barley also showed the effect of P deficiency could be recovered by resupplying barley with P (Carstensen et al. 2018a, b). As for *A. hypogaea*, it remains to be further studied whether the different P stress direction, strength and duration time can be restored by other physiological interventions (e.g., phytohormones or other nutrients), in addition to either resupplying P or decreasing the P supply.

Determining P stress thresholds to peanut plants with different degrees of damage in their leaf photosystems

How do we determine the different P stress thresholds (Suboptimal and Supraoptimal P versus Deficient and Toxic P levels) and ensure that these conceptual groups reflect realistic physiological and photo-biochemical characteristics in relation to available P nutrition? In order to attain this goal, three representative and conceptual groups were assigned: the exogenous OuP (Optimum P), SuP (Suboptimal and supraoptimal P) and EuP (Extremely unsuitable P including deficient and toxic P). Through PCA, other analyses of key plant physiological and photo-biochemical parameters (growth, leaf N and P concentrations, gas exchange, photosynthetic fluorescence) and data fitting, we found that the trade-off of the normalised Y(II) and Y(NO) might be the best proxy to determine the OuP, SuP and EuP groups, because they are the two key factors with significant positive and negative effects on PC1. In our study, the PCA was based on 23 plant physiological traits and this analysis accounted for 87.4% of the variance (Table 2 and Fig. 7). The first component (PC1) represented 75.5% of the variability. PC1 was successful in segregating the OuP, SuP and EuP groups (Fig. 7). The trade-off of the normalised Y(II) and Y(NO) is a potentially useful benchmark to allocate the different levels of P-fertilisation in *A. hypogaea.* According to the fitting curves of Y(II)s and Y(NO)s, when the P supply was <0.41 mM or > 1.72 mM, Y(NO)s value was greater than Y(II)s. This analysis implies that the trade-off of Y(NO) and Y(II) tended towards Y(NO), and the photosynthetic capacity of peanut leaves was severely inhibited with significant photodamage extending beyond PSII. With the P supply ranging from 0.41–1.72 mM, the trade-off of Y(II) and Y(NO) tended towards Y(II) (Fig. 8). The exogenous P supply of 0.41–0.8 mM was considered suboptimal; an exogenous P supply of 1.1–1.72 mM was considered supraoptimal for peanut growth. By fitting a non-linear curve between P supply and leaf P concentration (Fig. 9), we found that the theoretical optimum P supply of 0.93 mM corresponded with a total leaf P concentration of 8.9 mg P g^{-1} DW. The suboptimal P level range corresponded with leaf P concentrations of 4.8–8.1 mg P g^{-1} DW; likewise, the supraoptimal P supply corresponded with leaf P concentration of 9.9–12.2 mg P g^{-1} DW; the deficient and toxic P supply

corresponded with leaf P concentration of less than 4.8 mg P g^{-1} DW and greater than 12.2 mg P g^{-1} DW, respectively. In parallel, the total leaf P concentration of around 10.0 mg P g^{-1} DW was the critical level between sufficiency and toxicity in tomato plants (Benton and Jones 1998). Above this critical value, visual symptoms of P toxicity in plants would appear. Therefore, those authors suggested that P fertilisation can be adjusted in time through diagnosing foliar P sufficiency or deficiency in tomato or any other species (Benton and Jones 1998). In addition, other research also showed that P concentrations in organs of plants exposed to excessive P supply are probably much higher than the values of 12–20 mg P g^{-1} DW measured in whole leaves or shoots (Foote and Howell 1964; Rossiter 1952; Warren and Benzian 1959). High-yielding peanut is a typical phosphate-demanding crop, but there are few dedicated P-management/threshold guidelines for peanuts (Yu et al. 2016). At present, P application strategy for solution culture and soil culture of peanut is often based on a generic platform developed for legumes at large. Consequently, there are many instances of either over- or under-supply of P in hydroponics and field experiments, thereby affecting the environment and yield. Our current research provides timely and useful information about specific P requirements and a theoretical range across a series of P supplies. Our experiment was undertaken in hydroponics, and the soil environment is more complex. Therefore, it may not be applicable in all conditions, but it can give a reference for soil regulation and fertilisation. Based on this study, we aim to further consider other factors such as soil types, rhizosphere soil physico-chemical properties, and rhizosphere carboxylates.

Conclusions

Peanut growth and photosynthesis under P deficiency and toxicity were severely inhibited and associated with significant photodamage extending beyond PSII, ultimately reducing the linear electron flow to PSI. It was caused by the feedback of Calvin cycle limitation. The linear electron flow limitation had a negative impact on leaf photosynthesis under P deficiency and toxicity. For peanut, the optimum P supply ranges from 0.8–1.1 mM with the corresponding leaf P concentration ranging from 8.1–9.9 mg P g^{-1} DW. Although it was difficult to observe morphological symptoms under suboptimal and supraoptimal P supply, we managed to identify suboptimal and supraoptimal P concentrations, among the other P supplies, using the trade-off of the normalised Y(II) and Y(NO). The suboptimal P supply range (0.41–0.8 mM) corresponded with leaf P concentrations of 4.8–8.1 mg P g^{-1} DW; conversely, the supraoptimal P supply range (1.1–1.72 mM) corresponded with leaf P concentration of 9.9–12.2 mg P g^{-1} DW; the deficient and toxic P supply (<0.41 mM, >1.72 mM) corresponded with leaf P concentration of less than 4.8 mg P g^{-1} DW and greater than 12.2 mg P g^{-1} DW, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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