The effects of water regime on phosphorus responses of rainfed lowland rice cultivars

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• *Background and Aims* Soil phosphorus (P) solubility declines sharply when a flooded soil drains, and an important component of rice (*Oryza sativa*) adaptation to rainfed lowland environments is the ability to absorb and utilize P under such conditions. The aim of this study was to test the hypothesis that rice cultivars differ in their P responses between water regimes because P uptake mechanisms differ.

• *Methods* Six lowland rice cultivars (three considered tolerant of low P soils, three sensitive) were grown in a factorial experiment with three water regimes (flooded, moist and flooded-then-moist) and four soil P levels, and growth and P uptake were measured. Small volumes of soil were used to maximize inter-root competition and uptake per unit root surface. The results were compared with the predictions of a model allowing for the effects of water regime on P solubility and diffusion.

• *Key Results* The plants were P stressed but not water stressed in all the water regimes at all P levels except the higher P additions in the flooded soil. The cultivar rankings scarcely differed between the water regimes and P additions. In all the treatments, the soil P concentrations required to explain the measured uptake were several times the concentration of freely available P in the soil.

• *Conclusions* The cultivar rankings were driven more by differences in growth habit than specific P uptake mechanisms, so the hypothesis cannot be corroborated with these data. Evidently all the plants could tap sparingly soluble forms of P by releasing a solubilizing agent or producing a greater root length than measured, or both. However, any cultivar differences in this were not apparent in greater net P uptake, possibly because the restricted rooting volume meant that additional P uptake could not be converted into new root growth to explore new soil volumes.

Key words: *Oryza sativa*, rainfed lowland, phosphorus efficiency, root morphology, solubilization, rice cultivar.

INTRODUCTION

Rainfed lowland rice systems cover 54 Mha worldwide and produce 19 % of the world's rice (Bouman *et al.*, 2007). The average national yield across South and South East Asia is only 2·3 t ha^{-1} year^{-1}, but more rice will have to be produced from these systems to meet predicted demand for rice (Bouman *et al.*, 2007). This will be difficult because of the problems arising from the variability of water regimes in rainfed systems. The fields are bunded but not irrigated, so the soil may drain and re-flood at least once during the season. The resulting water stress is often compounded by nutrient deficiencies, particularly of phosphorus (P), because the soils are often inherently low in P and the solubility and plant availability of soil P are sensitive to changes in water regime (Haefele *et al.*, 2006).

Differences in P efficiency (i.e. the ability to capture soil P and utilize it in biomass production when other factors are not limiting) have been identified between rice genotypes in lowland and upland rice (Ismail *et al.*, 2007). The differences are often obscured by genotype × environment interactions under field conditions, particularly where there is uncontrolled flooding and drainage during the growing season (Fukai *et al.*, 1999). An ideal genotype would perform well under low soil fertility with fluctuating water regimes, but would also respond well to fertilizer application. To identify and transfer useful traits into broadly adapted genotypes it is important to understand the mechanisms involved and their genetic basis. However, it is proving difficult to explain observed differences in P efficiency between genotypes with known mechanisms. Various candidate mechanisms have been identified (Saleque and Kirk, 1995; Kirk *et al.*, 1999; Hoffland *et al.*, 2006), and Wissuwa has shown that in upland rice, mechanisms increasing the efficiency of roots to extract P from sparingly soluble pools are probably of primary importance (Wissuwa and Ae, 2001*a*, *b*; Wissuwa, 2003). However, a slight increase in root efficiency will lead to improved root growth and so to a positive feedback loop (Wissuwa, 2003); therefore, separating traits for root growth from those for ability to mobilize sparingly soluble soil P is difficult. Further, a rice cultivar able to acquire P efficiently in a flooded soil will not necessarily do so in a moist, oxidized soil because both the soil P chemistry and the plant nutrient relations are very different (Huguenin-Elie *et al.*, 2003).

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The present study tested the hypothesis that rice cultivar differences in their P responses differ between water regimes because of differences in P uptake mechanisms. This is done with measurements of growth and P uptake under different water regimes, and the results are analysed with a model allowing for the effects of water regime on soil P chemistry. Experiments were performed with small volumes of soil to maximize inter-root competition and rates of P uptake per unit root surface, and to minimize the effects of difference in root growth *per se*.

MATERIALS AND METHODS

The experiment was conducted in a phytotron at the International Rice Research Institute, Los Baños, Laguna, Philippines. Day and night temperatures were 27 and 21 °C, respectively, relative humidity $>70\%$, and light as ambient for a humid tropical wet season.

Plant material

Three traditional rice cultivars (*Oryza sativa* L., 'Sabita', 'Banla Phdao' and 'KDML105' from India, Cambodia and Thailand, respectively) considered tolerant of low P soil under rainfed lowland conditions were used, together with three modern cultivars (BG300, IR36 and IR31406-333-1) considered sensitive to low P soil under irrigated conditions (IRRI Problem Soils Germplasm Database, Quijano-Guerta *et al.*, 2002, www.iris.irri.org).

Soil

The experimental soil is a humic, kaolinitic clay (Tropaquept by USDA Soil Taxonomy; pH 4·5 in 0·01 M $CaCl_2$, CEC 48 $cmol_c$ kg^{-1}, base saturation 50 %, clay 640 g kg^{-1}, organic C 38 g kg^{-1}, Fe extractable with citrate-dithionite 63 g kg^{-1}) from a long-term experiment under lowland rice at Pangil, Laguna, Philippines, to which no P fertilizer had been added for several years. The soil was air-dried, ground to pass a 2 mm sieve and thoroughly mixed with monocalcium phosphate to give 0, 25, 50 or 100 mg P kg^{-1} (hereafter referred to as P_0, P_{25}, P_{50} and P_{100} soils). For all P additions the soil was further fertilized to give per kg: 90 mg N as urea, 90 mg K as K_2SO_4, 10 mg Mg as $MgCO_3$ and 5 mg Zn as $ZnSO_4$.

The amounts of resin-extractable P in the soil, [Resin-P], after equilibration at the experimental water regimes (next section) were measured by shaking the soils with excess Cl^- form anion exchange resin (BDH No 55164) at 1 $mmol_c$ of exchange capacity per g of soil, and recovering P from the resin. The values of [Resin-P] so obtained were 0·087, 0·221 and 0·350 mmol kg^{-1} in P_0, P_{100} and P_{200} moist soils, respectively, and 0·269, 0·391 and 0·647 mmol kg^{-1} in P_0, P_{100} and P_{200} flooded soils.

The soil moisture characteristic was determined by equilibrating 6 cm long, 6·6 cm diameter cylinders of the soil on tension tables as in the main experiment (next section) and measuring the soil moisture content by weight as the soil was drained from saturation (i.e. suction = 0 kPa) to field moist (6·5 kPa) in steps of 0·5 kPa suction. Equilibration

times were 48 h for 0–1 kPa, 96 h for 1·5–4·5 kPa and 120 h for 5·0–6·5 kPa.

Water regimes and plant growth

The fertilized soil was packed in 15 cm long, 6·6 cm internal diameter plastic cylinders to a bulk density of 0·90 kg dm^{-3} and equilibrated on tension tables at either field capacity (moist water regime) or saturation for 4 weeks before being planted. The tension tables comprised glass–microfibre filter paper disks connected to hanging water columns (for details, see Huguenin-Elie *et al.*, 2002). Redox potential measurements showed that the flooded soil was thoroughly reduced by the end of the incubation period.

Four 10-day-old seedlings grown in nutrient solution (Yoshida *et al.*, 1976) were transplanted into each soil cylinder and the soil surface covered with 1 cm of silica sand to reduce evaporation. The soil cylinders were kept on tension tables throughout the experiment and watered daily by injecting onto the soil surface 20 cm^3 of P- and Fe-free nutrient solution or 20 cm^3 of de-ionized water on alternate days. Constant and uniform water contents were maintained with this system for up to 42 d of plant growth (Huguenin-Elie *et al.*, 2002). Three water regimes were established: continuously flooded (volumetric soil water content, $\theta = 0.53$ dm^3 dm^{-3}) from 4 weeks before planting until harvest at 42 d; continuously moist at the maximum soil water holding capacity ($\theta = 0.30$ dm^3 dm^{-3}) for the same period; and flooded from 4 weeks before planting until 3 weeks after planting followed by moist until harvest. The experiment was arranged in a $3 \times 4 \times 6$ factorial split-split-plot design with four replicates: three water regimes as main plots, four P levels as sub-plots and six cultivars as sub-sub-plots. At harvest all cultivars were in the active tillering stage.

Plant analysis

The dry weight and P concentrations in plant tissues were measured at transplanting and at harvest. Roots were carefully washed free of soil over a 0·5 mm mesh and separated from shoots. Roots and shoots were dried at 70 °C for 72 h, weighed, and 300 mg portions digested in concentrated H_2SO_4 and H_2O_2 and analysed for P (Walinga *et al.*, 1995). Mycorrhizal infection of the roots was assessed by staining samples of fresh roots with trypan blue and examining under a microscope (Koske and Gemma, 1989).

Root development of cultivar KDML105

By visual inspection there were no major differences in root morphology between the cultivars, though there were differences between the water regimes. Therefore, root development was only analysed in detail for one cultivar (KDML105) in the three water regimes. Sixteen cylinders fertilized with 100 mg P kg^{-1} were prepared for each water regime and planted with seedlings of KDML105. Four cylinders for each water regime were harvested after 10, 20, 31 and 42 d. At harvest, the roots were washed free of soil and separated from the shoots, and root and shoot dry matter and P contents were measured. A portion of the fresh roots (half for the first

harvest and a tenth thereafter, determined on a fresh weight basis) were stained with methyl violet in 10 % ethanol and root length was measured using an image analysis system (Delta-T Devices Ltd, Burwell, Cambridge, UK). The roots were segregated into three categories according to diameter: (*a*) fine, 50–150 µm; (*b*) medium, 151–300 µm; and (*c*) coarse, > 300 µm.

The time-courses of root length development in KDML105 in the different water regimes together with the corresponding time-courses of P uptake were used to calculate the P inflow per unit root length over time. By analogy with the classic equations for net assimilation rate (Tinker and Nye, 2000, p. 284), the average inflow per unit root length between consecutive harvests at times t_1 and t_2 is:

$$I = \frac{(U_2 - U_1)(\ln L_2 - \ln L_1)}{(t_2 - t_1)(L_2 - L_1)} \quad (1)$$

where U = uptake per cylinder and L = root length per cylinder. The mean inflows were calculated over the intervals between harvests starting at transplanting.

Model of P uptake

The model of P uptake by a root system of increasing density developed by Huguenin-Elie *et al.* (2003) based on the classical model of Tinker and Nye (2000) was used. In this model, individual roots are treated as cylindrical sinks to which P moves by diffusion. The roots are not infected with mycorrhizas, and they do not release solubilizing agents or influence P solubility in the soil other than by removing P and thereby creating depletion zones across which P diffuses. The equations for P diffusion to an individual root are

$$\frac{\partial [P]}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(r D_{LP}\theta f \frac{d[P_L]}{dr}\right) \quad a < r < x, t \geq 0 \quad (2)$$

$$D_{LP}\theta f \frac{d[P_L]}{dr} = -\alpha [P_L] \quad r = a, t \geq 0 \quad (3)$$

$$D_{LP}\theta f \frac{d[P_L]}{dr} = 0 \quad r = x, t \geq 0 \quad (4)$$

where [P] and [P_L] are the concentrations of diffusible P in the whole soil and soil solution, respectively, D_{LP} is the diffusion coefficient of P in free solution, θ is the volume fraction of soil water, f is the diffusion impedance factor, α is the root absorbing power for P, r is radial distance, a is the root radius and x is the radius of the cylinder of soil influenced by the root.

The relationship between [P] and [P_L] is taken to be constant for a given soil water content and redox status, and is given by the soil buffer power for P, b_P:

$$d[P]/d[P_L] = b_P \quad (5)$$

Note that b_P, θ and f all vary with water regime. The radius of the cylinder of root influence x is obtained by assuming a regular parallel array of roots, which gives

$$x = 1/\sqrt{(\pi L_V)} \quad (6)$$

where L_V is the root length density. Root length density increases over time as the plants grow, and the mean inter-root distance, $x - a$, decreases correspondingly. The simultaneous spread of the P depletion zone around each root is given by Tinker and Nye (2000, p. 298)

$$x_d = 2\sqrt{(D_P t)} + a \quad (7)$$

where $D_P = D_{LP}\theta f/b_P$.

In the model these equations are solved numerically, allowing for the moving boundary at $r = x$ as L_V increases, as described by Huguenin-Elie *et al.* (2003).

Parameter values. Various simplifying assumptions were made in parameterizing the model. Later, how realistic these assumptions are is tested by way of a sensitivity analysis. First the geometry and dimensions of the root system were assumed to be independent of the water regime and P addition, and the following sigmoid relationship was used for the change in root length density over time, obtained by Huguenin-Elie *et al.* (2003) for KDML105 grown as here in P_{100} soil under all three water regimes:

$$L_V = \frac{L_{Vmax}}{1 + (L_{Vmax}/L_{V0} - 1)\exp(-L_{Vmax}k_r t)} \quad (8)$$

where L_{V0} and L_{Vmax} are the root length densities at $t = 0$ and $t = 42$ d, respectively, and k_r is a constant: $L_{V0} = 30$ dm dm^{-3}, $L_{Vmax} = 7258$ dm dm^{-3}, $k_r = 40·9 \times 10^{-6}$ d^{-1}. Also $a = 0·001$ dm.

Secondly, the root absorbing power α was assigned a value realistic for P-starved plants and it was assumed to be independent of the P concentration in solution at $r = a$, $[P_L]_a$. Commonly the relationship between the P influx and $[P_L]_a$ is described with a Michaelis–Menten equation: $F = F_{max}[P_L]_a/(K_M + [P_L]_a)$, hence $\alpha = F_{max}/(K_M + [P_L]_a)$, and if $[P_L]_a << K_M$, $\alpha = F_{max}/K_M$. Typical values for P-starved cereals are $F_{max} = 3–8 \times 10^{-4}$ µmol dm^{-2} s^{-1} and $K_M = 2–10$ µM (Tinker and Nye, 2000). Under the conditions used, $[P_L]_a << 2$ µM and so $\alpha \approx 1 \times 10^{-4}$ dm s^{-1}.

Thirdly, the initial concentration of plant-available P in the soil was assumed to be proportional to the amount of resin-extractable P; hence

$$[P]_{initial} = \gamma\rho[\text{Resin-P}] \quad (9)$$

where ρ is the soil bulk density (=0·9 kg dm^{-3}) and γ is a constant for a given water regime, which was obtained by fitting the model to the P uptake data. Further, from the

measurements of [Resin-P] at different additions of P,

$$[\text{Resin-P}] = \iota + \kappa[\text{P applied}] \qquad (10)$$

where ι and κ are constants for a given water regime. For the moist soil, $\iota = 8 \cdot 76 \times 10^{-5}$ mol kg^{-1} and $\kappa = 1 \cdot 32 \times 10^{-6}$ mol mg^{-1} ($r^2 = 0 \cdot 99$, $n = 3$), and for the flooded soil, $\iota = 2 \cdot 47 \times 10^{-4}$ mol kg^{-1} and $\kappa = 1 \cdot 89 \times 10^{-6}$ mol mg^{-1} ($r^2 = 0 \cdot 96$, $n = 3$).

Fourthly, it was assumed that the buffer power is independent of water regime and P addition. From measurements of P diffusion through the soil to a resin sink under the three water regimes (Huguenin-Elie *et al.*, 2003), a suitable value is $b_P = 1000$ dm^3 (solution) dm^{-3} (soil).

Also, for the moist soil, $\theta = 0 \cdot 4$, $f = 0 \cdot 3$, and for the flooded soil, $\theta = 0 \cdot 53$, $f = 0 \cdot 4$.

RESULTS

Effects of P fertilization and water regime on plant growth and P concentration

The soil water contents were uniform ± 5 % of θ throughout the soil volume at all times in all the treatments. The moisture characteristic of the soil in the experimental systems fitted the equation

$$\log(-\psi) = 2 \cdot 741 - 6 \cdot 636 \times \theta \quad r^2 = 0 \cdot 985, \quad n = 18 \quad (11)$$

where $\psi =$ soil matric potential and $\theta =$ volumetric water content. Hence for the moist soil treatment ($\theta = 0 \cdot 3$), which was the driest in the experiment, $\psi -5 \cdot 6$ kPa, which is well above the matric potential at which rice plants show water stress (Okada *et al.*, 2002). The plants were therefore not water stressed at any time in any of the treatments. Nor were there symptoms of mineral toxicities in the plants in any of the treatments, or of deficiencies other than P. Aluminium toxicity is not expected because, although the soil pH is quite low, the percentage base saturation is high (Materials and Methods).

Shoot dry weight. All the cultivars responded to P applications with increased shoot dry weight in all three water regimes (Fig. 1A). The cultivar rankings were similar across the water regimes and P levels, and the traditional cultivars (Sabita, Banla Phdao and KDML105) produced more shoot dry matter than the modern cultivars in all the water regimes and P levels. For all the cultivars, shoot dry weight was greater in the flooded-then-moist soil than in the moist soil but less than in the flooded soil. The increase in shoot dry weight per unit of P applied tended to decrease with increasing P application, especially in the flooded soil.

Shoot P concentration. Shoot P concentrations were below the accepted deficiency threshold for rice at the tillering stage (approx. 1 mg P g^{-1}; Dobermann and Fairhurst, 2000) in all the water regimes and P levels, except the P-fertilized flooded soil (Fig. 1B). The cultivar rankings were not much altered by water regime or P level, but were approximately the inverse of the shoot dry weight rankings, indicating that the traditional cultivars grew more with a similar amount of P taken up – the so-called dilution effect. The beneficial effect of flooding on shoot P concentration did not persist following drainage in the flooded-then-moist treatment.

Total P uptake. Total P uptake per cylinder varied with water regime and P application and between cultivars (Fig. 1C). In the flooded soil, the cultivar rankings were similar across P levels, with the cultivars considered 'P efficient' taking up approx. 25 % more P at the highest P addition than the modern 'inefficient' cultivars. In the moist soil, the cultivar differences were smaller in absolute and relative terms, and the rankings differed with P addition, the greater uptake by the traditional cultivars Sabita and Banla Bhdao at the highest P addition not being maintained without P addition. The differences in the flooded-then-moist soil were intermediate. At the end of the experiment, the amounts of P taken up by the plants far exceeded the amounts of resin-extractable P in the soil, implying that most of the P taken up was drawn from less soluble soil P pools.

Root dry weight, P concentration and mycorrhizal infection. Root dry weight was greater in the continuously flooded soil, especially in the traditional cultivars and KDML105 (Fig. 2A), and the relative increases with flooding were greater than the relative increase in shoot dry weight. Root dry weights were not significantly different across P applications, but the root:shoot dry weight ratio decreased with P application in all the water regimes (Fig. 2B). The root:shoot ratio was larger in the flooded than the moist soil at all P levels. For a similar increase in shoot P concentration, the decrease in root:shoot dry weight ratio was about 2-fold greater in the flooded than the moist soil.

Root P concentrations were small in all treatments and did not change significantly with P addition (data not shown). When shoot P concentration exceeded 1 mg P g^{-1}, root P concentration was generally less than shoot P concentration. However, under acute P deficiency, root P concentration tended to be maintained at the expense of shoot P.

No mycorrhizal infection was found in any of the water regimes. Mycorrhizal networks are disrupted by fluctuating water regimes under rainfed lowland conditions (references in Huguenin-Elie *et al.*, 2003).

Root morphology of KDML105. Root length increased during plant growth in all the water regimes but slowed after about 4 weeks when the volume of the pot evidently became limiting (Fig. 3A). In the flooded and flooded-then-moist soils, roughly 65 % of root length was as fine roots and the remaining 35 % was split equally between medium and coarse roots, and this distribution was constant over time. In the continuously moist soil, there was a greater proportion of medium roots, and the distribution changed over time. However, the variability among replicates was large (treatment mean coefficients of variation $4 \cdot 1 - 16 \cdot 3$ %) and the treatment differences were not statistically significant. In the flooded treatments, roots were mostly straight, whereas in the moist treatment they tended to be kinked. Very few new roots appeared after the soil was drained in the flooded-then-moist treatment.

There were significant differences in specific root length between the water regimes over time (Fig. 3B). After 4
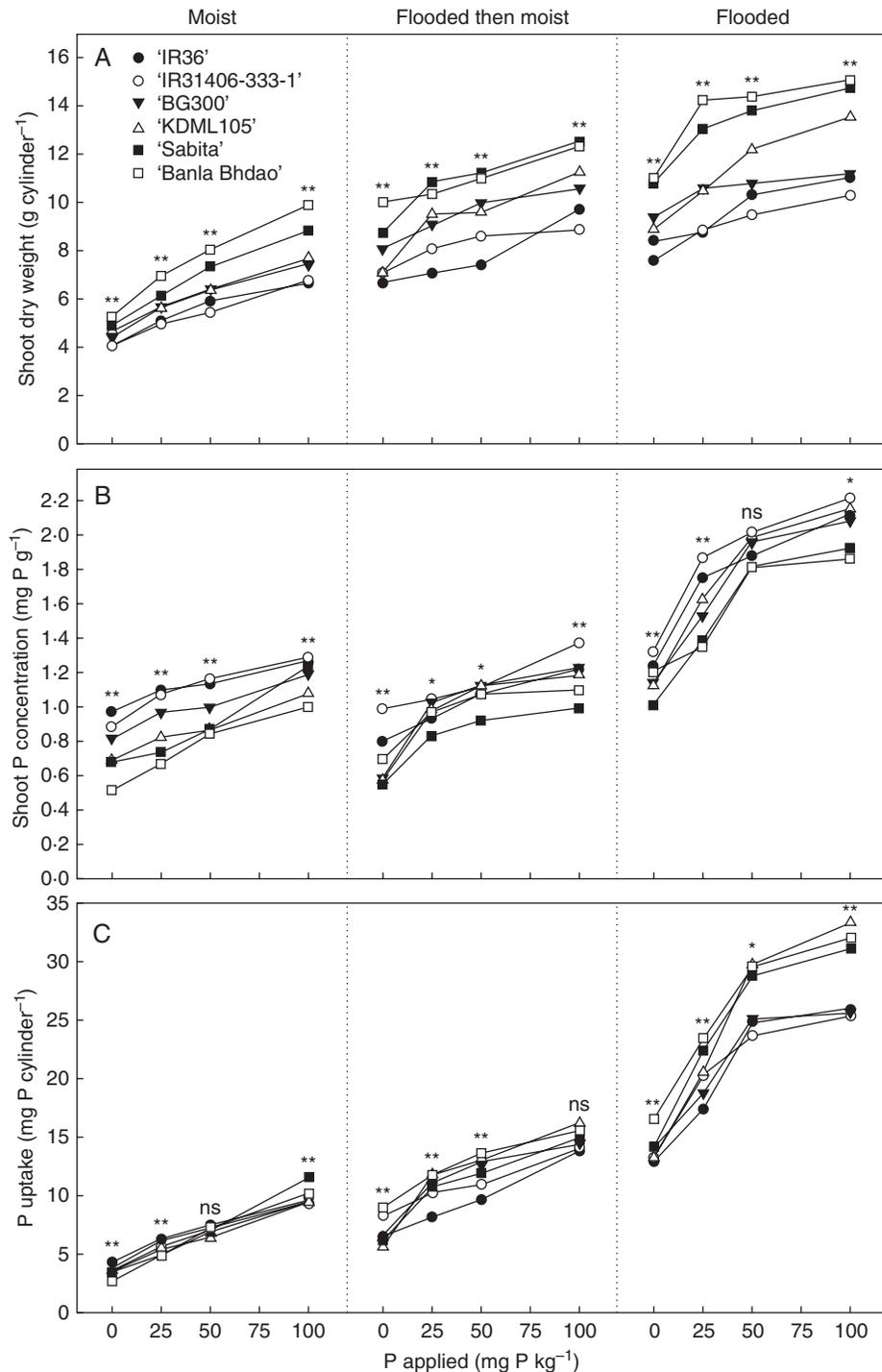
FIG. 1. Changes in (A) shoot dry weight, (B) shoot P concentration and (C) total P uptake with P applications in six rice cultivars grown under different water regimes. Time = 42 d after planting. Data are the mean of four replicates. $*P < 0.05$; $**P < 0.01$; ns = not significant.

weeks, specific root length was comparable in the moist and flooded-then-moist soils, but was far smaller in the flooded soil. The mean root radius was relatively constant over time (84 ± 5, 80 ± 7 and 92 ± 13 μm in the flooded, flooded-then-moist and moist soils, respectively). The large decreases in specific root length after 21 d therefore indicate large increases in mean root density. This may be explained

by increases in the proportion of fine, non-aerenchymatous roots. Coarse roots tend to be less dense because they are aerenchymatous, particularly in the flooded water regimes.

Figure 3C shows the changes in mean P inflow into the roots over time calculated from root length and P uptake using eqn (1). It shows that inflow was fairly constant over time in the flooded soil, but it strongly decreased after about 3 weeks in
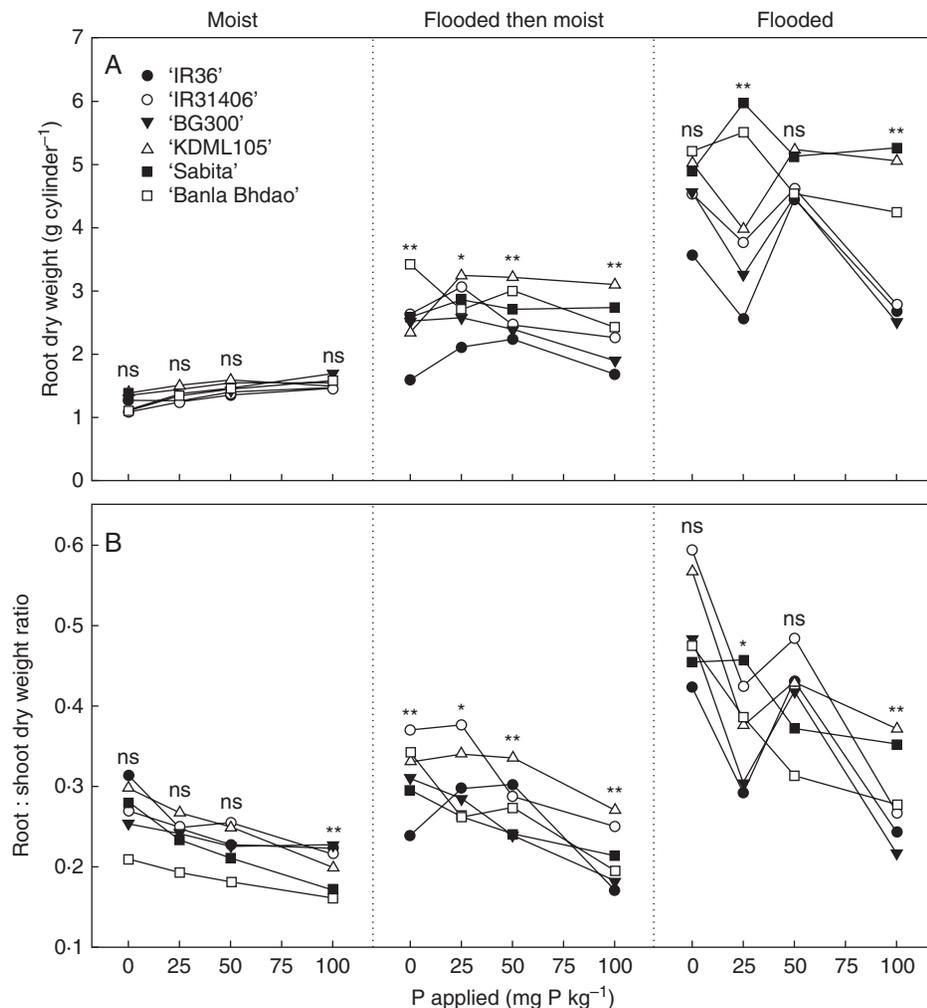
FIG. 2. Changes in (A) root dry weight and (B) root:shoot dry weight ratio with P applications for the experiment in Fig. 1. Data and symbols as in Fig. 1.

the moist and flooded-then-moist soils. Figure 3D shows the corresponding changes in mean inter-root distance, x, calculated with eqns (6) and (8), and the mean spread of P depletion around the roots, x_d, had the roots behaved independently of each other, calculated with eqn (7) and the values of θ, f, b_P and a given above. This shows that the mean inter-root distance was less than the spread of the depletion zone after about 3 weeks in the continuously moist soil and a few days earlier in the flooded soil. This matches the rapid decrease in P inflow per unit root length in the moist and flooded-then-moist soils. Figure 3C and D therefore indicates strong depletion of P in the moist and flooded-then-moist soils, consistent with decreased P solubility and diffusion. In the flooded soil, inflow was evidently not diminished as the soil P was depleted, at least in the P_{100} soil.

Indicators of P efficiency. In spite of the far greater total P uptake under flooded conditions, uptake per unit final root dry weight varied little between the water regimes because of the differences in root dry weight (data not shown). There were some differences between the cultivars in uptake per unit root weight. Under moist conditions, the highest values were observed in the modern cultivars BG300, IR36 and

IR31406-333-1 at P_0 and P_{25}. Similarly, in the flooded-then-moist soil, BG300 had the highest value at P_{50} and IR36 and BG300 the highest values at P_{100}. Finally, under flooded conditions, BG300, IR36 and IR31406-333-1 had the highest values at P_{100}.

Shoot weight per unit P uptake varied with water regime, P application and cultivars (data not shown). The values were greatest in moist soil and least in flooded soil, and in all water regimes it decreased strongly from P_0 to P_{50}, but changed little with further P additions. In the moist soil, Banla Phdao produced the greatest shoot weight per unit P uptake at all P rates and IR36 and IR31406-333-1 the least. The highest values were observed with Sabita in the flooded-then-moist soil and with Sabita and Banla Phdao in the flooded soil. IR31406-333-1 produced the least shoot weight per unit P uptake at P_0 in the flooded-then-moist soil and at P_0 and P_{25} in the flooded soil.

Model calculations

Figure 4 shows the calculated changes in P uptake with P application, and Fig. 5 shows the sensitivity of the model to its input
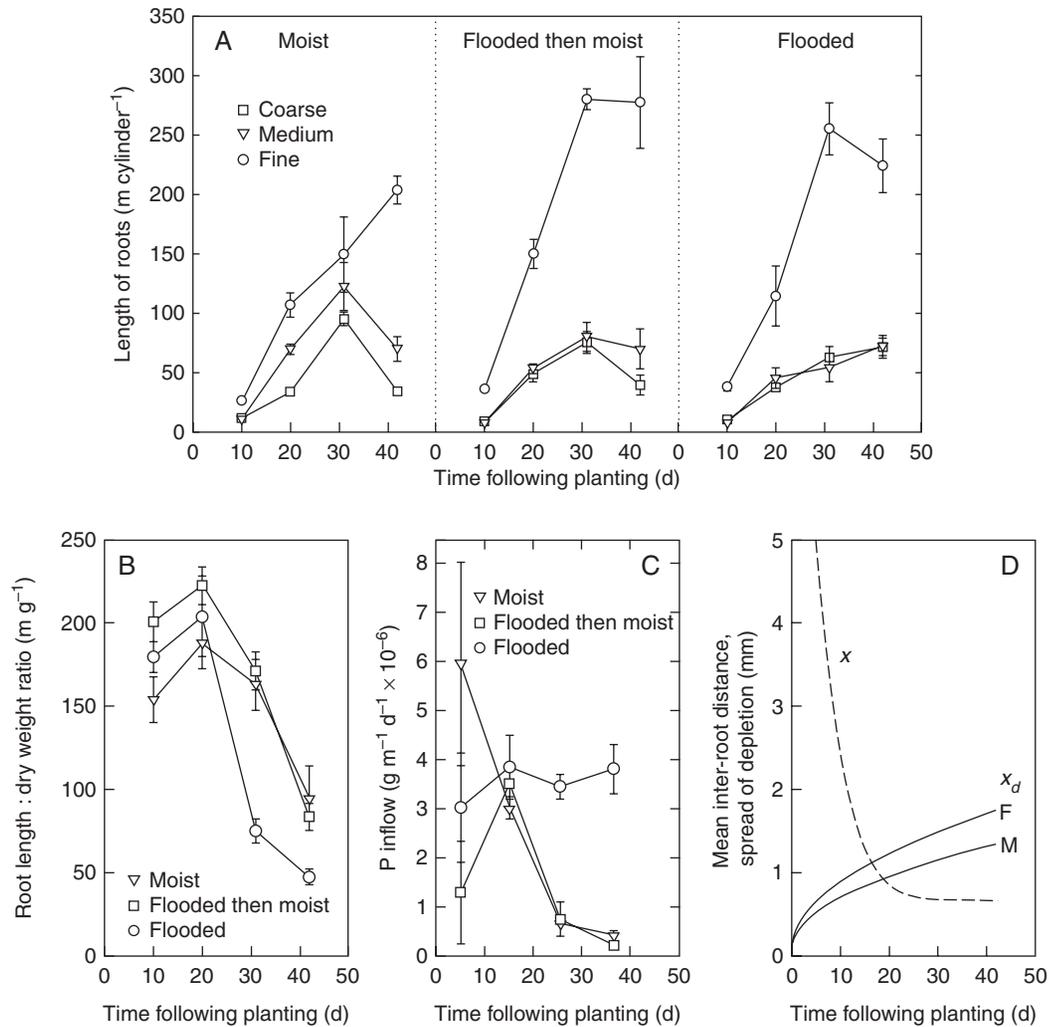
FIG. 3. Changes over time for cultivar KDLM 105 in soil with 100 mg P kg^{-1} in (A) lengths of different root classes (diameters: fine, 50–150 μm; medium, 151–300 μm; coarse, >300 μm), (B) specific root length, (C) P inflow per unit root length calculated with eqn (1) and (D) mean inter-root distances calculated with eqns (6) and (8) and spreads of depletion zones calculated with eqn (7) for flooded (F) and moist (M) soil. Data are means ± s.e. of four replicates.

parameters. Note, in Fig. 4, a distinction is made between the modern and traditional cultivars for the flooded soil because the differences in P uptake were substantial, but not for the moist soil. The results in Fig. 4 indicate the following.

(1) The apparent concentration of diffusible P from which the roots drew their P was approximately seven times the [Resin-P] in the moist soil and ten times the [Resin-P] in the flooded soil.

(2) In the moist soil, the slope of the uptake vs. P applied relationship was comparable with that calculated, but in the flooded soil it was steeper (at least at P applied <50 mg kg^{-1}).

(3) In the moist soil, the roots absorbed approx. 64 % of the estimated diffusible P and in the flooded soil approx 71 %; this value was independent of the ratio of apparently available P to [Resin-P], γ.

(4) The recovery of added P was about 12 % in the moist soil and 27 % in the flooded soil; this value varied with γ.

The sensitivity analysis in Fig. 5 was carried out at P_{25} because, in the flooded soil, the curvature of the uptake vs. P applied line at greater P additions indicates that uptake was no longer solely limited by P supply. The sensitivity analysis indicates that in both moist and flooded soil, uptake is insensitive to root radius and the root absorbing power, but sensitive to the maximum root length density, to the ratio of apparently available P to [Resin-P], and to the P buffer power.

DISCUSSION

The availability of P strongly limited growth at the smaller P additions in the flooded soil, and at all P additions in the other water regimes, as shown by increased shoot dry weight, shoot P concentration and total P uptake with P addition, and decreased root:shoot ratio. The increase in growth and uptake with P addition declined at the larger P additions, indicating P was increasingly non-limiting.
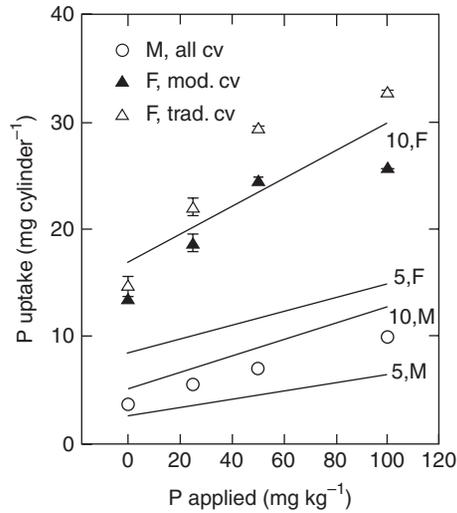
FIG. 4. Measured (means ± s.e.) and calculated (lines) changes in P uptake with P application. Letters indicate flooded (F) or moist (M) soil, and modern (mod.), traditional (trad.) or all cultivars (cv); numbers are ratios of apparently available P to resin-extractable P [γ in eqn (9)]. See text for other parameter values. Time = 42 d.

The cultivars all responded to water regime and P addition in broadly similar ways, and the cultivar rankings were largely independent of water regime and P addition. The differences were greatest in the flooded soil at the largest P addition, which is the treatment where P was least limiting. This suggests that the cultivar differences reflect differences in plant type and vigour rather than differences in P efficiency

per se. The traditional cultivars generally produced more growth for a given P uptake than the modern cultivars.

The smaller cultivar differences in P uptake under P-limited conditions, where the effects of efficient P acquisition should be most apparent, show that differences between the cultivars in depleting a restricted soil volume were small. Under unrestricted conditions, the effects of enhanced uptake per unit root surface may be amplified by resulting increases in root growth, so small improvements in uptake per unit root surface can produce large increases in net uptake (Wissuwa, 2003). Therefore, the failure to detect large differences in uptake per unit root surface between the cultivars does not necessarily mean they do not occur under field conditions. However, the original hypothesis cannot be corroborated with the present data.

The rest of the discussion is focused on the effects of water regime and P application averaged across the cultivars. The differences are considered in terms of the changes in P solubility and diffusion in the soil in response to water regime, and the changes in root growth and function.

Root growth and P uptake over time

Figure 3A and B shows that the proportions of roots of different classes and specific root length differed between water regimes, though total root length and mean root radius did not differ, at least for the cultivar KDML105. The relationship between root geometry and water regime depends on (*a*) the need for internal root aeration under flooded conditions and resulting changes in root morphology; (*b*) changes in the mechanical impedance of the soil with water regime; and (*c*)
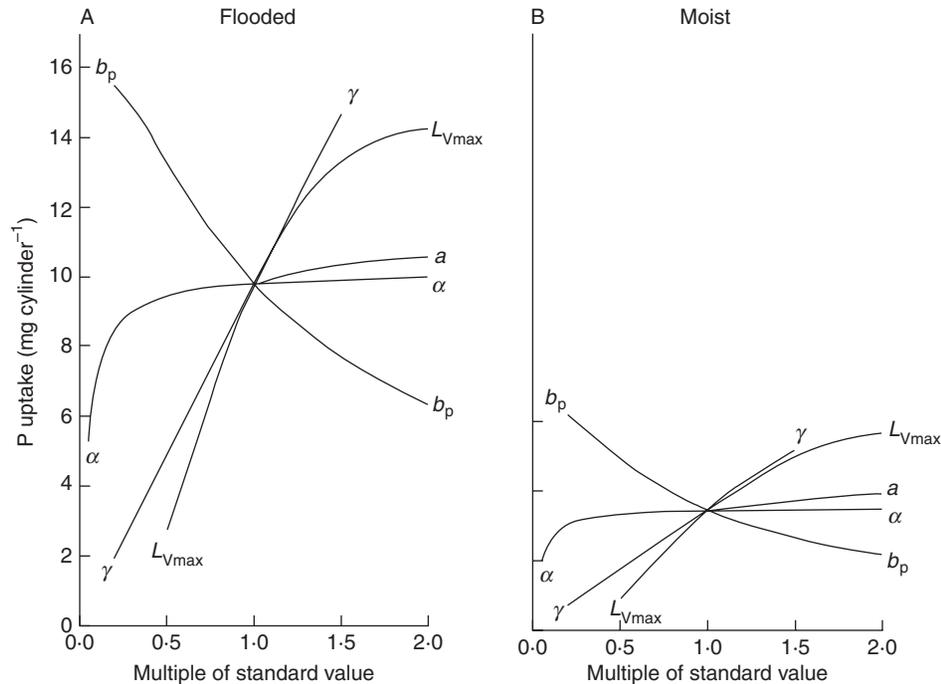

FIG. 5. Sensitivity of calculated P uptake to model parameter values in (A) flooded soil and (B) moist soil. The individual lines were obtained by varying the indicated parameter whilst keeping the other parameters constant. The *x*-axis give the parameter value relative to its standard value. The standard values are [$P_{applied}$] = 25 mg kg^{-1}, b_P (soil P buffer power) = 1000; γ (the ratio of apparently available P to resin-extractable P) = 5; L_{Vmax} (maximum root length density) = 7258 dm dm^{-3}; *a* (root radius) = 0·001 dm; and α (root absorbing power) = 1 × 10^{-4} dm s^{-1}. Time = 42 d.

changes in P solubility and diffusion with water regime. Kirk (2003) showed with a model of root aeration that the system of coarse, aerenchymatous, primary roots with gas-impermeable walls conducting O_2 to short, fine, gas-permeable laterals found in rice in flooded soil provides the greatest absorbing surface per unit aerated root mass. The greater proportion of fine roots in the flooded treatment is consistent with this. Under moist conditions, greater mechanical impedance will tend to produce a smaller proportion of fine roots (Thangaraj *et al.*, 1990), though lower P mobility in moist soil would tend to favour finer roots (Wissuwa, 2003). The greater proportion of intermediate roots in the moist soil seen here is consistent with this.

Figure 3C shows that P inflow per unit root length was fairly constant over time in the flooded soil, but it strongly decreased over time in the moist and flooded-then-moist soils. This would agree with decreased P solubility and diffusion in moist soil, and resulting strong depletion of available P over time, particularly once the depletion zones around neighbouring roots overlapped (Fig. 3D). In the flooded soil, P inflow per unit root length after 4 weeks was >4 times that in the moist soils. This suggests the 2·5- to 3-fold greater final P uptake in the flooded soil had more to do with differences in uptake per unit root length than differences in root morphology. In the following section, the modelling results are used to assess how far differences in P solubility and diffusion with water regime can explain differences in uptake per unit root length.

Plant-available P and the effects of water regime on P solubility and diffusion

The modelling results in Fig. 4 indicate that, in both the moist and flooded soils, the concentration of P that the roots were able to access was from five to ten times the concentration of [Resin-P]. Assuming [Resin-P] is a good measure of the P that is available to the roots if they just behave as passive sinks for P, this indicates the roots were able to tap less available forms of P, either by releasing a solubilizing agent or by producing a greater root length than measured, or both. The following arguments show that [Resin-P] is indeed a good measure of passively available P.

Huguenin-Elie *et al.* (2003) measured P diffusion from a block of the experimental soil at each of the water regimes to a layer of anion exchange resin – sufficient to maintain an effectively infinite sink for P. The passively available P can be estimated by fitting the measured time-courses of P accumulation in the resin to the following diffusion equation:

$$M_t = 2\sqrt{D_{LP}\,\theta f\, t/(\pi b_P)} \times [P]_\infty$$
$$= 2\sqrt{D_{LP}\,\theta f\, t/\pi} \times \sqrt{[P]_\infty [P_L]_\infty} \tag{12}$$

where M_t is the amount of P accumulated after time t and $[P]_\infty$ and $[P_L]_\infty$ are the initial concentrations of passively available P in the whole soil and in the soil solution, respectively. With values of $[P_L]_\infty$ measured in saturated pastes (approx. 1·1, 0·4 and 0·6 μM in the flooded, flooded-then-moist and moist soils, respectively; Huguenin-Elie *et al.*, 2003), the estimated values of $[P]_\infty$ are only a third to a sixth of [Resin-P]. Even though

saturated pastes tend to overestimate $[P_L]$ somewhat (Huguenin-Elie *et al.*, 2003), this shows that the passively available P was if anything smaller than [Resin-P]. It is therefore concluded that [Resin-P] is a satisfactory measure for the present purposes.

The P uptake was 2·5- to 3-fold greater in the flooded soil at the highest P addition. The increases, with flooding, in P solubility indicated by [Resin-P] and diffusion indicated by θf were both about 1·77-fold. Therefore, if uptake increases as $\sqrt{(\theta f)} \times$ [Resin-P] (cf. eqn 12), this accounts for most of the increased uptake in the flooded soil. However, the steeper slope of the measured uptake vs. P applied relationship in the flooded soil compared with that calculated (Fig. 4) suggests that the increases in uptake and inflow per unit root length were more than in proportion to the increase in P solubility. This indicates that the roots in the flooded soil were better at extracting less-soluble forms of P.

Limitations of the model

Although the average root dimensions were evidently similar across the water regimes, there were differences in the distribution of root classes, with a greater proportion of fine roots in the flooded soil (Fig. 3). Roose and Fowler (2004) made a model of solute uptake by branched root systems allowing for a distribution of root classes, though not for inter-root competition, and they found that inclusion of root branching gave a several-fold greater uptake of P compared with a model for the same total root mass but only a single root radius. This effect will be smaller in the present system because of the greater root length densities caused by the restricted soil volume and the consequently greater importance of inter-root competition. The sensitivity analysis in Fig. 5 indicates that uptake is insensitive to root radius *per se*.

The assumptions about soil P buffering may be more limiting. Following soil flooding, reductive dissolution of iron oxides and other solid phases on which P is sorbed will tend to decrease the soil P buffer power and increase the amount of soluble P (see Kirk, 2004, for a discussion of these effects). However, subsequent re-precipitation of ferrous iron in mixed ferrous–ferric hydroxides and carbonates will lead to an increase in buffer power and decrease in P solubility. In the estimates of b_P under the different water regimes from diffusion to a resin sink (Huguenin-Elie *et al.*, 2003), only approx. 50 % differences in b_P were found across the water regimes.

Huguenin-Elie *et al.* (2003) discuss potential solubilizing agents released from rice roots under different water regimes and calculate the magnitudes of their effects. In crude terms, the effect of a solubilizing agent will be to increase the amount of easily extractable P as represented by the parameter γ. In addition, the recovery of solubilized P by the root will depend on the relative spreads of the solubilizing agent and the P depletion zone away from the root: the further the spread of the solubilizing agent relative to P depletion, the less will be the recovery of solubilized P by the root. However, for the large rooting densities and small inter-root distances in the present experiments, recovery of solubilized P would be near complete.

CONCLUSIONS

Though there were differences in P responses between the cultivars, the cultivar rankings scarcely differed between the water regimes and P additions. Small volumes of soil were deliberately used in order to maximize inter-root competition and thereby the dependence of the plants on their ability to deplete soil P. So if there were any cultivar differences in attributes for this, they were very small. However, possibly such differences are amplified if the root volume is unrestricted and the additional P taken up can be converted into new root growth to explore new soil volumes.

For all the water regimes, P levels and cultivars, the soil P concentrations required to explain the measured uptake were several times the concentration of freely available P in the soil, suggesting the plants were able to tap sparingly soluble forms of P. The model sensitivity analysis indicates this may have been because they released a solubilizing agent or agents, or they produced a greater root length than measured, or both, but not because of a greater root absorbing power.

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