Limited effects of the soluble organic phosphorus fraction on the root phosphorus uptake efficiency of upland rice genotypes grown in acid soil

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Abstract

The root phosphorus (P) uptake efficiency (RE), defined as plant P uptake per unit root mass or root area, may contribute to the P efficiency of upland rice grown in acid, P-deficient soils. The identification of root traits conferring RE of rice has been compromised by the lack of attention given to P speciation when evaluating P-mining mechanisms. Here we disentangled the effect of soluble organic P (P_o) from that of total soluble P (P_T) on the RE in rice seedlings in acid soil. Six rice genotypes were grown for 21 days in P-deficient substrates, i.e. a sample of an acid mineral soil, an acid peat and a mixture thereof. Each of the three substrates were amended with different P doses, partially limed and incubated, yielding substrates with significant differences in total soluble P (0.004-0.41 mg P/L) and in the percentage of organic P in that pool (P_o/P_T , 0-57%). Plants showed a large growth response to the P addition. There was a significant genotypic variation in P uptake (0.9-1.5 mg P/plant) and RE (4.4-8.3 mg P/g root mass) under moderate P deficiency but these traits were unaffected by the P_o/P_T in the soluble fraction of the substrates. Along the same lines, phosphatase activity in the rhizosphere soil was unaffected by genotypes and did not explain the RE among all data ($R^2=0.17$, ns). A multiple regression model showed that the RE of rice seedlings was mainly affected by the inherent genotypic effects, the P_o/P_T and the total soluble P concentration in soil ($R^2=0.73$), while the genotype- P_o/P_T interaction only marginally improved the RE model ($R^2=0.78$). This suggests limited genotypic effects due to the better use of organic P. A root elongation test in the acid mineral soil that was either or not limed suggests that the differences in acid soil tolerance may play a larger role in the genotypic performance of RE than organic P utilization potential.

Keywords acid soil, P acquisition efficiency, rice, root efficiency, soil organic phosphorus

38 **1 Introduction**

39 Phosphorus (P) deficiency is the major constraint in upland rice production in highly weathered, acid P-
40 fixing soils, especially in small-scale farming systems due to the poor accessibility to inorganic P fertilizers
41 (Sanchez et al., 1997; Masso et al., 2017). Selecting for high P acquisition efficiency (PAE; P uptake capacity
42 of the plant under low P availability, expressed in mg P/plant) could be an entry-point for the resource-
43 poor farmers to reduce fertilizer requirements by expanding the soil P pool that rice plants can access.
44 There is a large genotypic variation in upland rice for PAE (Vandamme et al., 2016), which basically
45 attributes to the difference in root development under low P conditions and to lesser extent to root P
46 uptake efficiency (RE; plant P uptake per unit root mass or surface area) (Wissuwa and Ae, 2001; Mori et
47 al., 2016). With a modeling study, Wissuwa (2003) demonstrated that a small (i.e. around 30%) increase
48 of RE can result in as much as three-fold increase of PAE. This is achieved by a combined effect of increased
49 P uptake per unit root size (primary effect) and the subsequent root growth which amplifies the primary
50 P uptake effect on PAE (secondary effect). Currently, RE is observed as a combination of root hair
51 development and the plant capacity of P mining, i.e. taking up P from sparingly available sources (Mori et
52 al., 2016; Nestler and Wissuwa, 2016; Wissuwa, Gonzalez, and Watts-Williams, 2020). Improvement of RE
53 through an enhanced P mining capacity can be particularly useful in the acid P-fixing soils that are wide-
54 spread in upland rice production areas (Haefele, Nelson, and Hijmans, 2014).

55 It is well established that the root-induced mobilization of sparingly available P in the plant rhizosphere
56 involves roughly two mechanisms releasing either inorganically or organically bound P, directly or
57 indirectly by root exudate secretion (Richardson et al., 2011). Briefly, direct P mobilization by root
58 exudates are through 1) local acidification dissolving Ca-P complexes by proton release; 2) complexation
59 of Fe/Al species with organic anion release; 3) organic P mineralization by phosphatase release. Indirectly,
60 root exudates can also enhance rhizosphere P mobilization through plant-microbe interactions, as root

exudates attract soil microorganisms as readily available C sources (Spohn, Ermak, and Kuzyakov, 2013). The P mobilization in the rice rhizosphere has been suggested by many researchers; however, the breeding efforts to incorporate such traits has been complicated (see extensive review from Vandamme et al., 2016).

In highly weathered, acid soils rich in Fe/Al (hydr-)oxides, the majority of orthophosphate and generally abundant organic P species (i.e. inositol phosphates) are adsorbed to these oxyhydroxides and form stable complexes (Celi and Barberis, 2004; Guedes et al., 2016). Organic anions can mobilize these surface-complexed P, and root secretion of organic anions has been extensively studied for rice (Rose et al. 2013). However, the capacity of rice to release P from strongly bound Fe/Al-P complexes in such soils is questioned due to the low release rate (Wissuwa, Gonzalez, and Watts-Williams, 2020). In small-scale farming systems where OM amendments are widely applied as the alternative fertilizers, humic-bound phosphomonoesters can constitute a large fraction of total P (McLaren et al., 2015), with lesser extent phosphodiester (Turner and Leythem, 2004). These fractions, especially phosphodiester, are less strongly protected P species and hence more plant-accessible (Turner and Leythem, 2004; Gerke, 2015). Previous studies have suggested the significance of such relatively labile organic P fractions on rice P nutrition in highly weathered soils (Turner, 2006; Nishigaki et al., 2019). The upregulation of root extracellular phosphatase secretion in rice in response to P deficiency has been reported (Pariasca-Tanaka et al., 2009; Lu et al., 2016; Mehra et al., 2017; Gao et al., 2017). However, the effect of this trait on genotypic variation in rice PAE has been inconclusive (Rose et al., 2013). A number of constraints can be considered as follows: 1) limited number of tested genotypes; 2) complex nature of organic P species present in the tested soils, 3) overriding effect of more efficient microbial phosphatases on P mineralization, 4) soil components affecting the efficacy of phosphatases, and 5) limitation of measuring potential phosphatase activity as a surrogate of *in-situ* organic P mineralization capacity (Hedley et al.,

1994; Li et al., 2008; Quiquampoix and Mousain, 2004; Pariasca-Tanaka et al., 2009; Richardson et al., 2011).

Here, we assessed differences in upland rice genotypes in their capacity to acquire P from organic fractions in acid soils to explain their differences in RE. The selected rice genotypes were grown in the three acid, P-deficient substrates, i.e. a mineral soil, a peat soil and a mixture thereof. Each substrate was amended with different P doses, partially limed to an equal pH and incubated, yielding substrates with significant differences in total soluble P (P_T) and its organic P fraction (P_O/P_T) in the 0.01M $CaCl_2$ extracts as an index of P availability. Peat soil contains large amounts of organic P including humic-bound P and other readily hydrolysable organic P species (Shand and Smith, 1997; Turner and Newman, 2004), making it a relevant organic P source for rice plants to test organic P utilization under realistic soil conditions. The mineral soil was selected as an extremely organic P-poor substrate. By combining organic soil with mineral soil, this mixture soil has an intermediate property in all soil characteristics. The RE of tested genotypes was related to the concentrations of P and to the fractions of soluble P that is organic. The potential P mineralization capacity was measured as phosphatase activity in rhizosphere soil, which is a total sum of plant- and microbial phosphatase activity and the potential enzyme-deactivation effect of Fe/Al (hydro-)oxides in the soil. Our specific hypotheses were that: 1) Genotypic ranking in RE changes with an increasing fraction of organic P in the soil solution i.e. some genotypes stand out in RE when organic P is more dominant; 2) Genotypes previously identified with a high RE in the soil rich in organic P take up more P when the organic fraction of P is larger; 3) such genotypes show higher phosphatase activities in the rhizosphere soil than in the bulk soil, either because of higher secretion of phosphatase from their roots or from the rhizosphere microbial community.

2 Materials and methods

2.1 Plant materials

Six rice varieties (i.e. genotypes) including four *O. sativa* (*aus* subspecies: DJ 123, *indica* subspecies: Sadri Tor Misri, *japonica* subspecies: Chomrong Dhan and FOFIFA 172), one *O. glaberrima* (CG 14) and one interspecific variety between *japonica* and *O. glaberrima* (NERICA 4) were used in this experiment. All varieties were upland varieties except for Sadri Tor Misri, a lowland variety. In this experiment, NERICA 4 and FOFIFA 172 were used as the reference varieties since they have a large yield potential in the upland rice system and would be further improved for PAE by using candidate donor varieties. The four other varieties were used as potential high PAE donors with contrasting RE based on previous studies in P-deficient soils (Koide et al., 2013; Saito et al., 2015; Mori et al., 2016; Wissuwa, personal communication). Among the tested genotypes, Chomrong Dhan and FOFIFA 172 have not been tested for PAE but were added as potentially useful genotypes based on their grain yield performance under suboptimal OM-based fertilization scheme (Raboin et al., 2014).

2.2 Substrates

Two low-P acid soils with contrasting organic carbon content were selected for the mineral and organic substrates in this pot experiment. The top soils (0-20 cm) were collected from the unfertilized part of a farmer's upland field in Behenjy, Madagascar in April 2017 and a peat soil in Holsbeek, Belgium in October 2017 (Table 1). The soils were air-dried and sieved over 4 mm (mineral soil) and 1 cm (organic soil) for the pot experiment, and subsamples of both soils were further sieved over 2 mm to determine chemical properties (Table 1). Part of mineral and organic soils were mixed (50/50 by volume, i.e. 64/36 by weight) to create the third substrate, termed mixture. For the pot experiment, these substrates were moistened to 10% w/w with distilled water and pre-incubated for 1 month at 25°C. This pretreatment was done in

order to restore microbial activity and bring it to a more stable condition (i.e. stable respiration after 3 weeks; Vermeiren, personal communication).

2.3 Pot experiment

The pre-incubated substrates were limed to $\text{pH}_{\text{CaCl}_2}$ 4.5 (Solid:Liquid = 1:10) with 0.5 M $\text{Ca}(\text{OH})_2$ suspension at the following doses: 0.37 (mineral), 1.30 (mixture) and 2.59 (organic) g of $\text{Ca}(\text{OH})_2$ per kg substrate. The substrates were thoroughly mixed and incubated for 7 days at 25°C for equilibration. This pH was selected in order to limit the depletion of organic P from the CaCl_2 -extrable P pool, which will be explained later in this section. Together with lime, other nutrients were also applied as blanket as follows: 33.3 mg K/kg substrate (as KCl), 20.8 mg Mg/kg and 27.5 mg S/kg (as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.83 mg Fe/kg (as $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), 0.83 mg Mn/kg (as $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$), 0.83 mg Zn/kg (as ZnCl_2), 0.83 mg Cu/kg (as $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), 0.083 mg B/kg (as $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$), and 0.083 mg Mo/kg (as $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$). Nitrogen (N) was applied as NH_4NO_3 to have a final amount of 100 mg N/kg available over the entire experiment, taking into account the N mineralization of the mixture and the organic substrates. Both substrates showed the same low N mineralization rate (i.e. 0.9 mg N/kg/day) by 1 M KCl substrate extraction (Houba et al., 1989).

After 7 days of incubation of the limed and fertilized soils, each of the three substrates were further fertilized with P, yielding three levels of P availability. The three P availability levels, with P concentrations expressed as mg P/L in the 0.01M CaCl_2 extract, were established as follows: low P (i.e. native substrate P, 0.004-0.03 mg/L), medium P (i.e. critical P concentration, 0.1 mg/L; Hue and Fox, 2010), and high P (i.e. saturated amount of P, 1 mg/L). The high P level was used to determine the growth potential of each genotype in this experiment, through a presumably adequate supply of P. In the medium and high P treatments, different amounts of soluble P were applied as KH_2PO_4 to obtain the same total P concentration in 0.01 M CaCl_2 extracts of all substrates (P_T), based on the P sorption isotherm of limed

substrates using the protocol described by Six, Smolders and Merckx (2013) (Figure S1, supplementary information). To obtain that isotherm, a fresh, limed substrate sample of 2-g air-dry equivalent was incubated for 16h in 20 ml of 0.01 M CaCl_2 solution with increasing doses of KH_2PO_4 (i.e. 0-2000 mg P/kg). Samples were then centrifuged at 2000 g for 10 minutes, and the subsample of supernatant was filtered through a 0.45 μm membrane filter for ICP-MS. The total P concentration in the filtered supernatants was measured by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS; Agilent 7700x, Agilent Technologies, Inc.) with a Limit of Quantification (LOQ) equal to 3 μg P/L. The inorganic P concentration was measured by spectrophotometry after color reaction with malachite green (Van Veldhoven and Mannaerts, 1987) and measuring at a wavelength of 630 nm (Lambda 25, Perkin Elmer Inc.) with a LOQ of P_i equal to 8 μg P/L. The organic P (P_o) in solution was calculated by subtracting the inorganic P concentration (P_i , malachite green) from the total P (ICP-MS), i.e. $\text{P}_o = \text{P}_T - \text{P}_i$. Hereby we assumed that the organic P was negligible in the P measured by the malachite green method (Van Moorlegghem et al. 2011). To achieve 0.1 and 1 mg/L of solution P concentration by 0.01 M CaCl_2 extraction, the following P doses were applied: 250 mg and 600 mg P/kg to the mineral, 250 mg and 650 mg P/kg to the mixture, and 250 mg and 800 mg P to the organic substrate, respectively. The substrates were wetted with distilled water to 82-89% of the water holding capacity (i.e. WHC; 35, 44, 59 L/kg oven-dry soil for mineral, mixture and organic substrate). The moist substrates were filled in PVC tubes (4 cm diameter $\times$ 25 cm height) with the bottom part closed with a nylon mesh (pore size 2 mm) to have the same soil volume (i.e. about 2 cm below the edge). This resulted in 250, 195, 147 g air-dry equivalent/pot for the mineral, mixture and organic substrate, respectively. The substrate was capillary-wetted from the cup set at the bottom of the tube. Rice seeds were immediately sown after wetting the substrates.

Before planting, rice seeds had been sterilized with 0.5% NaOCl solution and pre-germinated for 3-6 days at 25°C in a wet paper towel soaked in 1 mM CaSO_4 solution. Two seeds were sown in each tube and covered with black plastic pellets to avoid rapid moisture evaporation from the substrate surface. When

most seedlings of each genotype reached 5 cm (i.e. 3-5 days after sowing, DAS), the young seedlings were thinned to have one plant per tube for a uniform growth in each genotype. The plants were grown for 21 days in the growth chamber with a 12/12h day/night cycle with 25/20°C day/night temperature, 75% relative humidity and the photosynthetic photon flux density of 721.3 $\mu\text{mol}/\text{m}^2/\text{s}$. The substrate moisture content was adjusted every day by adding distilled water to compensate for the weight loss of the tubes. In order to evaluate non plant-induced organic P mineralization (i.e. P mineralization without direct/indirect effect of root exudates) during growth period, substrate columns without plant (i.e. no-plant control) were incubated for 10 days side by side to the plants. Genotypes, substrates and P treatments were completely randomized with 3 replicates, and the position of the PVC tubes was changed every week.

After 10 days, fresh substrate samples were collected from the no-plant control and kept at 4°C until analysis. At plant sampling at 21 DAS, shoots were cut and roots were carefully extracted from the substrate as follows. The whole substrate columns were gently pulled out of the PVC tubes and the majority of substrate removed from the roots by hand. The roots were subsequently shaken and the remaining thin layer (i.e. approximately 2-4 mm) of substrate adhering to the roots was defined as rhizosphere substrate (Veneklaas et al., 2003). That fraction was collected separately from the bulk substrate by gently rubbing roots with fingers with clean nitrile gloves. The rhizosphere and bulk substrate samples were immediately stored in a cool box before transferring to a freezer set at -25°C. Roots were further cleaned by rinsing with tap water on a 1 mm sieve.

2.4 Plant and substrate measurements

197 Fresh substrate samples from the no-plant control were extracted in 0.01M CaCl₂ solution for 2h (Houba
198 et al., 2000) to measure inorganic and organic P concentrations as described for the method of P sorption
199 isotherm.

200 Shoot and root dry weights were recorded after drying at 70°C for 2 days. Whole shoot samples were
201 digested with nitric acid at 120°C in the block digester (DigiPREP MS, SCP Science Co.), and whole root
202 samples digested with 40% aqua regia at room temperature (i.e. around 20-23°C) after muffling at 550°C
203 for 4h (Benton Jones, Jr. and Case, 1990). The P concentration of both plant parts were determined with
204 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; iCAP 7000 series, Thermo Scientific
205 Inc.). The P acquisition efficiency was expressed as the absolute value of shoot P content (mg P/plant)
206 obtained at low P level. Root P uptake efficiency (RE) was calculated as total plant P content divided by
207 root dry weight in mg P/g root dry weight. The RE was only calculated for the suboptimal P level (i.e. low
208 P level) because P uptake at the optimal P level (i.e. medium and high P levels) is controlled by other
209 factors than the efficiency in P uptake (Vandamme et al., 2016). At the low P level, RE was only calculated
210 when detectable P uptake was observed (i.e. total P content > seed P content).

211 Phosphatase activity in the rhizosphere substrate of each genotype in the low P treatment was
212 determined with a microplate assay using 4-methylumbelliferone (MU) which is measured fluorimetrically
213 after releasing phosphate from its phosphorylated form, based on Marx et al. (2001) and DeForest (2009)
214 after some modifications described below. Triplicate samples of the bulk substrate in each substrate
215 treatment were included. Random genotypes were selected for bulk substrate sampling based on the
216 assumption that the difference in genotype only influences rhizosphere substrate. Due to possible
217 variation of measured activity among pots, the activity of the collected bulk substrates was compared
218 with the rhizosphere substrates of the corresponding pots (i.e. n=3). To measure phosphatase activity of
219 selected substrate samples, 400 mg of frozen substrate sample was mixed with 40 ml of sterile 25 mM

acetate buffer adjusted to pH 4.5 and homogenized by vortex for 2 minutes. While one sample was being weighed, the other samples were kept in a polystyrene box filled with ice cubes. All samples were handled within 20 minutes before the buffer was mixed to avoid thawing. Aliquots of 200 μ l of the obtained homogenates were pipetted into the solid black microplate making three analytical replicates for each sample, and subsequently mixed with either 50 μ l of increasing concentration of sterile MU solution (i.e. standard curve: 0, 2.5, 5, 10, 25, 50 μ M) or 0.5 mM sterile 4-methylumbelliferyl phosphate (MUP) solution. A standard curve was made for each substrate sample due to the difference in background quenching. A duplicate of the buffer control (i.e. buffer without substrate mixed) was applied for each microplate set to calculate autohydrolysis of MUP. NaOH was not added to increase pH because the fluorescence was sufficiently detectable in the preliminary test. Microplates were incubated at 25°C for 45 minutes in the dark condition. The fluorescence intensity was measured using a microplate fluorimeter (Fluoroskan Ascent FL, Thermo Fisher Scientific Co.) with 355 nm excitation and 460 nm emission wavelength. The phosphatase activity was calculated as μ mol MU released from MUP per g oven-dry substrate (105°C) per hour incubated. The MU stock solution was made 1 day before assay and renewed after 3-4 days to prevent degradation (Deforest, 2009). The MU working standard series and the MUP solution were also made 1 day before assay, but freshly made every time. It should be noted that the comparison of absolute values is only valid within this experiment due to possible sample degradation after long storage of frozen substrate samples (i.e. over 6 month).

2.5 Statistical analysis

All data were subjected to analysis of variance (ANOVA) using JMP software (JMP pro 14, SAS Institute Inc.). The standard least square model was applied to examine the effect of genotype (G), substrate (S), P level (P) and their interactions on P uptake and RE of the tested rice plants. Tukey's honestly significant

difference (HSD) test with a threshold of $P < 0.05$ was used to compare means of genotypes within the $S \times P$ treatment. Dunnet's test with a threshold of $P < 0.05$ was used to compare means of the phosphatase activity in the rhizosphere substrate with that of the bulk substrate.

A multiple linear regression model was tested in order to assess the effect of genotype, available P and substrate organic P fraction in the CaCl_2 extract on P uptake expressed by shoot P content and RE. The model reads:

$$\gamma = (\beta_0 + \beta_{0, \text{genotype}}) + \beta_1 * (P_T) + (\beta_2 + \beta_{2, \text{genotype}}) * (P_O/P_T) + \beta_3 * (P_T) * (P_O/P_T)$$

where γ represents either P uptake or RE, and $\beta_0, \beta_1, \beta_2, \beta_3$ are regression coefficients which are adjusted to reflect the variation of *genotype* (categorical variable) by applying genotype-specific corrector values ($\beta_{i, \text{genotype}}$; i is 0 or 2). P_T represents the total P concentration (mg P/L) in the 0.01M CaCl_2 substrate extract at the day of transplanting, and P_O/P_T represents the percentage (%) of organic P to the total P concentration in the substrate extract. Data from the mineral low P substrate was excluded because of no detectable P uptake, and all data from the high P level was also excluded due to high P supply exceeding plant requirement. The threshold of $P < 0.05$ was applied to evaluate the significance of model parameters.

3 Results

3.1 Change of inorganic and organic P concentration in the substrate extract without plants

The total P concentration in the CaCl_2 extract of the substrates and the organic fraction of P in that extract was significantly affected by the P level, the substrate and their interactions (Table 2). At the day of planting, the total P concentration (P_T) ranged between 0.004-0.04 mg P/L among substrates at the low P level, with the mineral substrate severely P-deficient compared to the organic and the mixture substrate (Table 2). The organic fraction of the P in solution (P_O/P_T) ranged between 0-40% at this P level. The organic

P fraction decreased in the mineral substrate as well as in the mixture substrate at the medium P level to roughly 20%, and it was mostly replaced by inorganic P in all substrates at the high P level. In the organic substrate, the organic P fraction was about 50% at the medium P level. After 10 days of substrate incubation, the total P concentration at the low P level was higher in the organic substrate compared to the two other substrates (Table 2). The fraction of organic P was depleted in two substrates except for the organic substrate. In this substrate, the organic P fraction decreased by 10% while the absolute concentration of organic P was unchanged.

3.2 Plant growth response to the increasing P level

The plants demonstrated a large growth response to the increasing P availability from the low P to the medium P level (Figure 1). Especially the plants grown in the mineral substrate displayed a sharp increase in biomass accumulation and P uptake, with total biomass and P content roughly increasing 10- and 80-fold, respectively. In the mineral substrate, plant growth was extremely limited at the low P level, as derived from extremely small biomass and the high root/shoot ratio in response to severe P deficiency stress. The average P content of 0.04 mg P/plant suggested that the plant P uptake from this substrate was negligible because it was almost as low as the average seed P content of 0.05 mg P/plant (data not shown). Data from the mineral substrate at the low P level was, therefore, excluded from further analysis of the genotypic variation in PAE and RE. In the mixture and the organic substrates, the plants experienced moderate P deficiency stress, as derived from consistent root/shoot ratio and relatively small decrease (30-34%) in total biomass compared to the severely P-deficient mineral substrate (88% decrease. In this experiment, solution P concentration at the medium P level was confirmed to be adequate for the maximal growth. Going from medium P to high P level, biomass accumulation stagnated while P uptake kept increasing indicating luxury uptake (Figure 1). Therefore, in a further comparison of genotype

performance related to P uptake and RE, we focused on the low P (P deficiency) and the medium P (optimal P) levels. The shoot P data from the low P mineral substrate and three high P substrates is available in Table S1 (Supplementary information).

3.3 Effect of the organic P fraction on the genotypic ranking of PAE and RE

A significant genotypic variation in the shoot P content (mg P/plant) was observed in all substrates at both P levels (Figure 2). At the medium P level, all genotypes experienced lower P uptake in the organic substrate compared to the other substrates. The multiple regression model for shoot P content showed a negative effect of the organic P fraction and a positive effect of its interaction with the total P, suggesting that the shoot P content was controlled by the P availability in the soil solution (Table 3). A significant effect of the genotype on P uptake was also found (Table 3) whereas the difference in the organic P fraction did not affect the genotypic ranking of shoot P content (Figure 2). This suggests little genotypic effects on the utilization of organic P. Chomrong Dhan and Sadri Tor Misri were among the best genotypes in terms of both P uptake potential (i.e. P uptake at the medium P level) and PAE (i.e. P uptake at the low P level), while CG 14 was among the lowest (Figure 2). Chomrong Dhan also showed high relative shoot P content at the low P level compared to the medium P level in both substrates (Figure 2). NERICA 4 and FOFIFA 172 (i.e. modern varieties) showed high relative shoot P content in the organic substrate at low P supply but the P uptake potential of these genotypes was among the lowest compared to the others.

There were significant genotypic effects on RE in the organic and the mixture substrate at the low P level (Figure 3). A poor correlation was observed between PAE and root biomass in both substrates ($R^2=0.004-0.01$, data not shown). This may indicate that the genotypic performance in RE was not only reflecting the difference in root size but also the other factors e.g. plant capacity to use organic P in the substrate solution. However, the multiple regression model for RE revealed the similar trend as in shoot P content

(Table 3). The total soluble P and its organic P fraction in soil explained 60% of the variation in RE, and adding the genotype increased that to 73%; however, adding the interaction between genotype and organic P fraction only increased that to 78% of the variation (details not shown). This illustrates that the effect of genotypes is only very poorly related to the different use of organic P in the soil solution. In both substrates, Chomrong Dhan and NERICA 4 showed high RE (Figure 3). There was no effect of genotype on potential rhizosphere phosphatase activity, i.e. the tested genotypes showed a similar level of potential phosphatase activity in the rhizosphere soil, regardless of substrates (Table 4). A higher phosphatase activity was found in the rhizosphere than in the bulk soil for the mixture substrate only (Dunnett's test, $p < 0.01$). This was possibly due to a large within-replicate variation in the organic substrate, because no significant difference was found between the mixture and the organic substrate in both rhizosphere and bulk substrate samples ($p > 0.05$; data not shown). No correlation was observed between RE and the rhizosphere phosphatase activity (Figure S2, supporting information).

4 Discussion

4.1 Effect of soil organic P fraction on the genotypic variation of upland rice in RE in acid soil

Our model system did not achieve consistent P concentrations across the substrates at the low P level, because of extreme P deficiency of the mineral substrate (Table 1). This problem constrained the complete establishment of a gradation of organic P fractions at the low P level. Nevertheless, the remaining substrates achieved two distinct levels of organic P fraction size and hence allowed the comparison of the effect of organic P utilization on rice PAE and RE. At the low P level, the use of peat soil achieved a two-fold difference in organic P supply compared to the other substrates in the absence of plants as intended (Table 2). Higher P mineralization was observed in this peat soil, possibly due to the enhanced organic matter mineralization in the peat soil at the aerobic condition of this experiment (Qiu and McComb, 1994)

and microbial cell lysis after air-drying and re-wetting of the peat soil upon pre-incubation (Turner et al., 2003).

In our system, no P uptake was observed in the low P mineral substrate despite its reasonable level of total P (Table 1, Figure 1). In this type of soils, the presence of Fe/Al (hydr-)oxides controls P availability for rice plants by its strong binding with P, giving it strong protection from mobilization to become plant-available (Nishigaki et al., 2019). The obtained results support our assumption that rice plants have limited capacity to draw P from sparingly available P in highly weathered, acid soils without any amendment, as also suggested by some authors (Rose et al., 2013; Wissuwa, Gonzalez and Watts-Williams, 2020; De Bauw et al., 2020).

In both of the mixture and organic substrates, genotypes showed roughly two-fold variation in PAE as well as RE (Figure 2 and 3). The variation in PAE was not explained by the genotypic capacity to maintain larger root size to explore for plant-available P in the substrate, supporting the observations by Mori et al. (2016) and Wissuwa, Gonzalez and Watts-Williams (2020) at medium P deficiency level. In their studies, the variation in RE was not completely explained by the difference of either root hair development or arbuscular mycorrhizal colonization (Nestler and Wissuwa, 2016; Wissuwa, Gonzalez and Watts-Williams, 2020), suggesting other P-mining mechanisms involved. We hypothesized that the fraction of organic P in the substrate CaCl_2 extract changes the genotypic ranking in PAE as well as RE because some genotypes may possess higher capacity to utilize the soil organic P pool at the low P availability. However, the organic P fractions in the two substrates neither affected PAE nor RE and genotypic ranking was consistent between the two substrates; the multiple regression model confirmed this lack of strong genotypic effect on organic P utilization (Table 3, Figure 2 and 3). The organic P pool was specifically considered as labile organic P which does not require unrealistically high secretion of organic anions to break protection of metal-organic-P complexes but still requires hydrolyzing agents before P uptake. Hedley et al. (1994)

observed that 6-week-old rice seedlings increased potential phosphatase activity at the rhizoplane, while the organic P (i.e. NaOH-P₀) fraction was unaffected. Their conclusion on rice organic P utilization was hampered by the extremely severe P deficiency limiting plant P uptake to almost solely from seed P. Little genotypic variation in rhizosphere phosphatase activity (Table 4) and the lack of relationship with the variation of RE (Figure S2) suggested that the tested rice genotypes had a similar potential to utilize organic P, with plant-microbial interactions as well as phosphatase-soil interactions taken into account. Taken together, the rice genotypes tested here were likely to take up a similar amount of P from soil organic P fraction, both in terms of potential and actual capacity in organic P mineralization. Little difference between the rhizosphere and the bulk substrate suggests that their organic P mineralization capacity was also very low (Table 4). This result is in line with the recent observation by Rakotoson, Holz and Wissuwa (2020), using three rice genotypes with contrasting PAE.

A limitation is that this study was still based on a small number of tested genotypes; i.e. the obtained results may have merely attributed to the lack of (existing) genotypes with higher capacity. A limited number of studies suggests the existence of such genotypes (Li et al., 2008; Alogaidi, 2013), although the difference in soil conditions should be taken into account as further discussed in the following subsection. The interpretation of this study is also limited to the early growth stage (i.e. 21 days). Since rhizosphere soil is greatly affected by rhizodeposition, the rhizosphere soil enzyme activity logically varies with plant growth stage, which affects both quality and quantity of root exudates (Ma et al., 2018). A general increase of enzyme activity during the vegetative stage has been observed for a number of crop species, as corresponding to the temporal dynamics of root exudation (Ma et al., 2018). For rice, Ge et al. (2017) observed an increased activity of acid phosphatase during 30 days of growth. This result implies higher importance of organic P utilization at later growth stage of rice. This study hence leaves an opportunity for further research to test larger panel of rice genotypes before excluding organic P utilization from breeding target.

381

382 **4.2 Potential effect of soil acidity on organic P utilization and RE of genotypes**

383 Our study targeted acid soils (i.e. pH <5.5), where the interactions between P deficiency and soil acidity
384 affects plant growth. We deliberately limed the soil only weakly for reflecting the field relevance. At this
385 level of soil acidity (i.e. pH 4.5), acidity stresses (especially aluminum (Al) toxicity) was likely to affect rice
386 PAE and RE by inhibiting root growth, reducing uptake of water and hence P_i (Shavrukov and Hirai, 2015).
387 Compared to the mixture substrate, higher P_i in the organic substrate did not lead to higher plant biomass
388 or P uptake at both low P and optimal P levels (Table 2, Figure 1). Moreover, the obtained genotypic
389 ranking of PAE and/or RE for some tested genotypes considerably differed from previous studies (Koide
390 et al., 2013; Saito et al., 2015; Mori et al., 2016; Wissuwa, Gonzalez and Watts-Williams, 2020), all of which
391 tested at pH >5.0. In our study, DJ 123, CG 14 and Sadri Tor Misri were identified as less efficient varieties
392 in both PAE and RE (Figure 2 and 3), while these genotypes consistently showed high PAE in the previous
393 studies on various soil types. Especially DJ 123 showed high RE in andosols that are rich in organic P (Mori
394 et al., 2016; Wissuwa, Gonzalez and Watts-Williams, 2020). De Bauw et al. (2020) recently demonstrated
395 that the differences in Al tolerance of upland rice genotypes could explain genotypic variation in response
396 to farmyard manure application in highly weathered, acid soils. In our system, the effect of soil acidity on
397 plant P uptake may have preceded the potential effect of organic P utilization.

398 To confirm that the tolerance to soil acidity may explain genotypic variation in PAE and RE, five rice
399 genotypes (except for Sadri Tor Misri because of seed shortage) were further tested for the acidity
400 tolerance by testing root elongation in the mineral soil that was either unlimed (i.e. pH 4.3) or limed to
401 optimal levels (i.e. pH 5.5, 5.9, 6.3; Table S2, supporting information). The detailed experimental settings
402 of this test can be found in the supporting information. The root length of the genotypes were reduced
403 by 20-40% in the most acid soils compared to the optimal ones (Table S2). This test showed that the acid

soil tolerance ranked FOFIFA 173> FOFIFA 172> Chomrong Dhan> CG 14> DJ 123> NERICA 4. Hence, this revealed the tendency of low to intermediate tolerance of tropical varieties versus high tolerance of Malagasy highland varieties. The obtained acidity tolerance ranking does not fully correspond to the PAE ranking; however, this may explain high performance of Chomrong Dhan and the low performance of DJ 123 in our system (Figure 2 and 3). The latter genotype was previously identified as high RE genotype with better root hair length and density in a P-deficient andosol that was not extremely acid, i.e. pH 5.7 (Nestler and Wissuwa, 2016). These root hair traits were likely not advantageous in the acid soil as root hairs are highly sensitive to acidity stresses including both Al^{3+} and H^{+} toxicity (Shavrukov and Hirai, 2015). In line with the findings of De Bauw et al. (2020), our study highlights the importance of acidity stresses in the genotypic comparison of P deficiency tolerance.

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Disclosure statement

The authors declare that there is no conflict of interest.

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599 **Table 1.** Soil properties of two acid, low-P soils with contrasting SOC contents sampled for the pot experiment

Soil	Collection site	Soil type [†]	pH [‡]	SOC [§] %	P-Total [¶] mg/kg	P-Ox mg/kg	Al-Ox mg/kg	Fe-Ox mg/kg	Mn-Ox mg/kg	P _i -CaCl ₂ [#] mg/L	P _o -CaCl ₂ [#] mg/L
Mineral	Behenjy (Madagascar)	Ferralsol	4.0	3.3	620	29.3	2670	1720	21.9	<0.01	<0.01
Organic	Holsbeek (Belgium)	Histosol	3.7	13.9	3690	3020	1820	48200	68.8	0.02	0.06

600 [†]Soil classification (WRB, 2015). [‡]pH (1:10) determined in 0.01M CaCl₂. [§]Soil organic carbon determined with elemental analyzer after combustion at 900°C.601 [¶]Total P determined in aqua-regia digests (Houba et al., 1989). ^{||}Ammonium-oxalate extractive P, Al, Fe and Mn (Schwertmann, 1964). [#]Readily available P
602 determined in 0.01M CaCl₂ soil extracts at the solid:liquid ratio of 1:10 (Houba et al., 2000). P_i (i.e. inorganic P) determined with Malachite green (Van Veldhoven
603 and Mannaerts, 1987) and P_o (i.e. organic P) determined by subtracting value of Malachite green from that of ICP-MS.

Table 2. The total P concentration (P_T) and the organic P fraction (P_O/P_T) in 0.01 M CaCl_2 extracts of three substrates at 0 and 10 days after planting at three added P levels. ***= $p<0.001$; **= $p<0.01$; *= $p<0.05$; ns= $p>0.05$, in the effect of P treatment, substrate and its interaction.

Substrate	Low P		Medium P		High P	
	$P_T^{\S}$ mg/L	$P_O/P_T^{\P}$ %	P_T mg/L	P_O/P_T %	P_T mg/L	P_O/P_T %
<i>Day 0[†]</i>						
Mineral	0.005	0	0.09	20	0.16	0
Mixture	0.020	36	0.07	25	0.41	1
Organic	0.044	43	0.13	55	0.38	11
<i>Day 10[‡]</i>						
Mineral	0.004	0	0.02	0	0.15	0
Mixture	0.018	0	0.04	15	0.12	0
Organic	0.058	31	0.08	18	0.22	3
Effect	P_T		P_O/P_T			
	Day 0	Day 10	Day 0	Day 10		
P treatment (P)	***	***	***	***		
Substrate (S)	***	***	***	***		
P × S	***	***	*	***		

[†]Substrate sampled before planting (n=2). Note that samples are only duplicated due to insufficient amount of the substrate sample available. [‡]Substrate sampled from no-plant control after 10 days of incubation (n=3). [§]Total P determined with ICP-MS. [¶]Percentage of the organic P (P_O determined by subtracting P_I , the inorganic P, from P_T) over the total P concentration. This is set to 0 if $P_O < \text{LOQ} = 0.008 \text{ mg/L}$.

611 **Table 3.** Parameter estimates and probability significance of the multivariate regression coefficients of the shoot P content and the root P uptake efficiency for
612 six genotypes in three substrates at two P levels (i.e. the low and the medium P levels). Note that values from the mineral substrate at low P level were removed
613 from this model due to no detectable P uptake, and all values from the high P level were also excluded due to high P supply exceeding plant requirement. Total
614 model R² is 0.88 for shoot P content and 0.78 for RE.

Parameter [†]	Estimate	Genotype-specific corrector value					
		NERICA 4 (N4)	FOFIFA 172 (F172)	DJ 123 (DJ)	Sadri Tor Misri (STM)	CG 14 (CG)	Chomrong Dhan (CD)
<i>Shoot P content</i>							
θ_0	3.52***	-0.30***	-0.24**	0.06 ns	0.45***	-0.30***	0.33***
θ_1	13.0***						
θ_2	-0.07***	0.005 ns	0.003 ns	-0.003 ns	-0.02*	0.01 ns	-0.0001 ns
θ_3	0.84***						
<i>Model R²=0.88</i>							
<i>Root P uptake efficiency</i>							
θ_0	13.3***	1.14**	-1.22**	-2.06***	0.54 ns	-0.21 ns	1.80***
θ_1	46.7***						
θ_2	-0.21***	0.005 ns	0.05 ns	0.05 ns	-0.12***	-0.003 ns	-0.009 ns
θ_3	0.78 ns						
<i>Model R²=0.78</i>							

615 [†] $\gamma = (\theta_0 + \text{genotype-specific corrector value}) + \theta_1 * (P_T) + (\theta_2 + \text{genotype-specific corrector value}) * (P_O/P_T) + \theta_3 * (P_T) * (P_O/P_T)$, where P_T is the total P
616 concentration (mg P/L) in the substrate extract in 0.01M CaCl₂ (data of day 0) and P_O/P_T is the percentage (%) of organic P fraction to the total P concentration in
617 the substrate extract in 0.01M CaCl₂. ***=p<0.001; **=p<0.01; *=p<0.05; ns=p>0.05. The P_O/P_T values of the P_O<0.008 mg/L was replaced by zero.

Table 4. Comparison of six genotypes for mean acid phosphatase activity in the rhizosphere and bulk substrates in two substrates at the low P level (i.e. native substrate P: <0.05 mg P/L in CaCl₂ extracts). Standard deviation is indicated after mean values (n=3 for the rhizosphere substrate of each genotype, and n=3 for each of the bulk substrate). ***=p<0.001; **=p<0.01; *=p<0.05; ns=p>0.05, for the variation in phosphatase activity among either genotypes (upper) or between rhizosphere and bulk substrates (lower). Substrates were prepared to have a range of SOC content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). Note that the mineral substrate was not included due to no detectable P uptake.

	Phosphatase activity μmol MU/g substrate/h incubation	
	Mixture	Organic
<i>Genotype rhizosphere</i>		
NERICA 4	0.31±0.07	0.30±0.11
FOFIFA 172	0.24±0.04	0.21±0.05
DJ 123	0.35±0.04	0.20±0.09
Sadri Tor Misri	0.20±0.03	0.20±0.04
CG 14	0.32±0.07	0.19±0.05
Chomrong Dhan	0.27±0.03	0.23±0.09
Effect	ns	ns
<i>Rhizosphere vs bulk substrate</i> [†]		
Rhizosphere	0.31±0.03	0.15±0.04
Bulk	0.20±0.01	0.23±0.06
Effect	**	ns

[†]Three pots were randomly sampled (i.e. regardless of genotype or replicate) to compare rhizosphere and bulk substrates. The phosphatase activity in the bulk substrates were assumed to be equal for all genotypes based on the assumption that the rhizosphere extent is within 4 mm (i.e. the approximate distance used for the rhizosphere substrate sampling). Because of a large variation within both bulk and rhizosphere samples in the organic substrate, this seemingly decreasing activity in the organic rhizosphere substrate compared to the bulk can be considered sampling effect rather than actual decrease.

Figure 1. Overview of the plant response to three P levels in three substrates. Bar plots represent the mean value of biomass (upper) and total P content (lower) with standard error of six genotypes within each substrate × P treatment (n=3). The biomass is divided into shoot (meshed) and root (solid). ***, p<0.001; **, p<0.01; *, p<0.05; ns, p>0.05, in the effect of genotype (G), P level (P), substrate (S) and their interactions on total biomass, root/shoot ratio (R/S) and total P content. Substrates were prepared to have a range of SOC content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). P levels were adjusted based on the target P concentration in the CaCl₂ extracts as: low P, native soil P at <0.05 mg P/L; medium P, 0.1 mg P/L; high P, 1 mg P/L.

Figure 2. Comparison of mean shoot P content of six genotypes in the mixture and the organic substrate at the low P and the medium P levels. Error bar indicates standard error of mean shoot P content of genotypes within each substrate $\times$ P treatment ($n=3$). Same alphabet indicates no significant difference among genotypes within each substrate $\times$ P treatment (Tukey's HSD, $p>0.05$). Genotypes are ordered from the highest (top) to the lowest mean value (bottom) for each substrate $\times$ P treatment. Substrates were prepared to have a range of SOC content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). P levels were adjusted based on the target P concentration in the CaCl_2 extracts as: low P, native substrate P at <0.05 mg P/L; medium P, 0.1 mg P/L; high P, 1 mg P/L. Note that values from the mineral substrate at low P level were removed from this figure due to no detectable P uptake, and all values from the high P level were also excluded due to high P supply exceeding plant requirement.

Figure 3. Comparison of mean root P uptake efficiency (RE) of six genotypes in the mixture and the organic substrate at the low P level (i.e. native substrate P at <0.05 mg P/L in CaCl_2 extracts). Error bars indicate standard error of mean RE of genotypes within each substrate $\times$ P treatment ($n=3$). Same alphabet indicates no significant difference among genotypes within each substrate $\times$ P treatment (Tukey's HSD, $p>0.05$). Genotypes are ordered from the highest (top) to the lowest mean value (bottom) for each substrate $\times$ P treatment. Substrates were prepared to have a range of SOC content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). Note that values from the mineral substrate at low P level were removed from this figure due to no detectable P uptake.

Supplementary information

Root elongation test screening for acid soil tolerance of rice genotypes

The acidity tolerance was assessed in a five-day root elongation test with an acid, mineral soil that was either or not limed. The root elongation of six genotypes (i.e. NERICA 4, FOFIFA 172, FOFIFA 173, DJ 123, CG 14 and Chomrong Dhan; Sadri Tor Misri was not included due to the seed shortage) was measured in the mineral soil (Table 1, main text) adjusted to four pH levels: native soil pH (i.e. pH 4.3) and three higher levels (i.e. pH 5.5, 5.9, 6.3). The soils were pre-incubated at 10% w/w for 2 days and limed with the suspension of 0.5M Ca(OH)₂ at doses equivalent to 1.11, 1.48 and 2.22 g Ca(OH)₂ per kg soil, respectively, for three limed soils. The soils were subsequently incubated at 16% w/w for 42 days at 25°C with periodic mixing to bring the soil to an equilibrium. The KH₂PO₄ was applied to all soils at the rate of 100 mg P/kg just before sowing, and soil moisture content was brought to 76% of water holding capacity (i.e. WHC, 0.35 L/kg oven-dry soil) with distilled water. The moist soils were transferred to 50-ml Falcon tubes, and three replicate tubes were used per genotype × liming treatment. In each tube, three pre-germinated seeds (see Materials and Methods in the main text) were sown and covered with black plastic pellets to avoid fast water evaporation. Plants were grown in the incubation room set at 20°C with the photosynthetic photon flux density of 238 μmol/s/m². Distilled water was added to bring soil moisture to approximately 70% WHC at 4 days after sowing (DAS). On the next day (i.e. 5 DAS), plants were gently extracted from the soil and roots were rinsed with tap water. The length of the longest root of each seedling was measured in a tray placed over a mm-scale sheet. The mean value of the longest roots of the three seedlings in each tube was used as the treatment replicate, i.e. n=3 for each genotype × liming combination.

The soil pH and soluble Al concentration were recorded at the start of the experiment. Fresh soil samples (equivalent of 3 g air-dry soil) were extracted in 15 ml of 1 mM CaCl₂ solution for 24 hours end-

over-end (20°C, 26 rpm). Samples were centrifuged for 10 min at 2000 g, and the subsample of supernatant was filtered through 0.45-µm membrane filter for ICP-MS. The remaining extracts were used for pH measurement, following the procedure described in Materials and Methods (main text). The Al concentrations were 2730 (pH 4.3), 7680 (pH 5.5), 20600 (pH 5.9), and 38700 µg Al/L (pH 6.3). At the native soil pH, the dominant soluble Al species was likely highly phytotoxic Al³⁺, while at higher pH less toxic species e.g. Al(OH)₂⁺ (Ryan and Delhaize, 2017).

A log-logistic model was used to describe the plant root growth response to soil pH for each genotype (Figure S3). The model reads:

$$Y = \frac{a}{\{1 + \exp((pH_{20} - pH) * b - \ln(4))\}}$$

where *a* is the maximum root length when pH is optimal (cm), pH₂₀ is the soil pH at which the root length is 20% lower than the maximal root length and *b* is the constant which determines the slope of the response line (Table S2). The relative root length (% cm/cm) at the lowest pH (i.e. 4.3) was calculated as the ratio of the mean root length in the unlimed soil to the maximal root length (parameter *a*) and the estimated length at pH 4.3 from this model. The data show that NERICA 4 is the most sensitive to acid soils and that FOFIFA 173 is the least sensitive; the 95% confidence intervals of the relative root length at the lowest pH do not overlap and indicate significant difference.

Reference

Ryan, Peter, and Manny Delhaize. 2017. "Adaptations to Aluminium Toxicity." In *Plant Stress Physiology*, edited by Sergey Shabala, 2nd ed., 178–200. Oxfordshire, United Kingdom: CABI.

713 **Table S1.** Comparison of mean shoot P content (mg P/plant) of six genotypes in the mineral substrate at the low P level and three substrates at
714 the high P level (n=3). ***=p<0.001; **=p<0.01; *=p<0.05; ns=p>0.05, for the variation in shoot P content. Data followed by the same character
715 within each column indicate no significant difference among genotype within each substrate × P treatment (Tukey's HSD, p>0.05). Substrates
716 were prepared to have a range of SOC content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). P levels were
717 adjusted based on the target P concentration in the CaCl₂ extracts as: low P, native substrate P at <0.05 mg P/L; medium P, 0.1 mg P/L; high P, 1
718 mg P/L.

Genotype	Low P level [†]	High P level [‡]		
	Mineral	Mineral	Mixture	Organic
NERICA 4	0.02 c	5.29	4.47 b	3.71 c
FOFIFA 172	0.03 b	4.13	3.37 b	3.79 c
DJ 123	0.04 a	4.68	5.16 ab	4.48 bc
Sadri Tor Misri	0.04 a	5.62	7.07 a	6.62 a
CG 14	0.02 c	5.11	4.41 b	4.42 bc
Chomrong Dhan	0.03 b	5.32	5.29 ab	5.02 b
Effect	***	ns	***	***

719 [†]Reflects a difference of seed P content due to no detectable P uptake (i.e. shoot P content < seed P content).[‡]Reflects a difference of the
720 genotypic potential in accumulating P in aboveground biomass due to luxury P uptake (i.e. P availability > plant requirement). Also reflects a
721 difference in acidity tolerance because P is not the growth-limiting factor at this P level.

722 **Table S2.** Parameter estimates for the log-logistic curve of root length (cm) of six genotypes in response to the pH change (n=3 for each of the
723 four pH treatments: pH 4.3, 5.5, 5.9, 6.3). The relative root length to the maximum at the optimal pH (*a*) was estimated for each genotype at pH
724 4.3 with 95% confidence interval (CI). Standard error of the estimated parameters is indicated in brackets. Rice plants were grown for 5 days in
725 the mineral soil adjusted to four pH levels by liming without P deficiency stress (i.e. 100 mg added P/kg).

Genotype	Parameter estimates [†]				Relative root length (%)	
	a	b	pH ₂₀	RMSE	Estimate at pH 4.3 (%)	95% CI
NERICA 4	7.9 (0.3)	3.59 (0.01)	4.6 (0.1)	0.98	60 (7)	44-75
FOFIFA 172	10.9 (0.1)	1.15 (0.34)	4.5 (0.2)	0.70	77 (4)	68-85
FOFIFA 173	9.8 (0.8)	1.58 (3.04)	4.1 (0.3)	0.69	85 (4)	76-95
DJ 123	9.5 (0.3)	1.83 (0.00)	4.7 (0.1)	0.87	67 (5)	55-79
CG 14	9.7 (0.3)	2.72 (2.70)	4.5 (0.2)	0.55	70 (3)	63-77
Chomrong Dhan	9.9 (0.5)	2.27 (3.57)	4.3 (0.2)	0.69	79 (4)	70-89

737 [†] $Y = a / \{1 + \exp((pH_{20} - pH) * b - \ln(4))\}$, where *a* is the maximum root length when pH is optimal (cm), pH₂₀ is the soil pH at which the root length is
738 20% lower than the maximal root length and *b* is the constant that determines the slope of the response line. Root mean square error (RMSE) of
739 the model is indicated for each genotype.

740

742 **Figure S1.** P sorption isotherm of three substrates. Substrates were prepared to have a range of SOC
 743 content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). The sorbed P to
 744 the solid phase (mg P/kg) after 16h of incubation in the 0.01M CaCl₂ background was fitted to the
 745 Freundlich equation ($Y = K_f \cdot [Solution\ P]^n$), where K_f and n are model parameters. Root mean square
 746 error (RMSE) of the fitted curve is indicated for each substrate. Two P application rates were selected to
 747 have approximately 0.1 mg P/L (medium P) and 1 mg P/L (high P) based on the above sorption curve for
 748 each substrate.

749

750 **Figure S2.** Relationship between root P uptake efficiency (RE) and phosphatase activity at the low P level
 751 (i.e. native substrate P, <0.05 mg P/L in CaCl₂ extracts) in the mixture and the organic substrate.
 752 ***=p<0.001; **=p<0.01; *=p<0.05; ns=p>0.05. Substrates were prepared to have a range of SOC
 753 content as: mineral, low SOC (3%); mixture, medium SOC (7%); organic, high SOC (14%). Note that the
 754 mineral substrate was not included due to no detectable P uptake.

Figure S3. Relationship between root length (cm) and pH of six genotypes at 5 DAS. The root length data was fitted to the log-logistic curve (see Table S1). Rice plants were grown in the mineral soil adjusted to four pH levels (i.e. 4.3, 5.5, 5.9, 6.3) by liming without P deficiency stress (i.e. 100 mg added P/kg).