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Critical Review

Enhancing Phytate Availability in Soils and Phytate-P Acquisition by Plants: A Review

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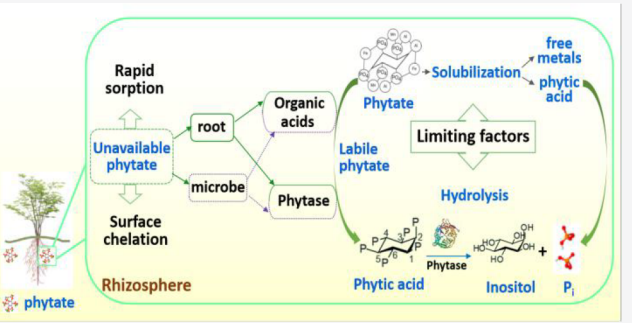
ABSTRACT: Phytate (*myo*-inositol hexakisphosphate salts) can constitute a large fraction of the organic P in soils. As a more recalcitrant form of soil organic P, up to 51 million metric tons of phytate accumulate in soils annually, corresponding to ∼65% of the P fertilizer application. However, the availability of phytate is limited due to its strong binding to soils via its highly-phosphorylated inositol structure, with sorption capacity being ∼4 times that of orthophosphate in soils. Phosphorus (P) is one of the most limiting macronutrients for agricultural productivity. Given that phosphate rock is a finite resource, coupled with the increasing difficulty in its extraction and geopolitical fragility in supply, it is anticipated that both economic and environmental costs of P fertilizer will greatly increase. Therefore, optimizing the use of soil phytate-P can potentially enhance the economic and environmental sustainability of agriculture production. To increase phytate-P availability in the rhizosphere, plants and microbes have developed strategies to improve phytate solubility and mineralization by secreting mobilizing agents including organic acids and hydrolyzing enzymes including various phytases. Though we have some understanding of phytate availability and phytase activity in soils, the limiting steps for phytate-P acquisition by plants proposed two decades ago remain elusive. Besides, the relative contribution of plant- and microbe-derived phytases, including those from mycorrhizas, in improving phytate-P utilization is poorly understood. Hence, it is important to understand the processes that influence phytate-P acquisition by plants, thereby developing effective molecular biotechnologies to enhance the dynamics of phytate in soil. However, from a practical view, phytate-P acquisition by plants competes with soil P fixation, so the ability of plants to access stable phytate must be evaluated from both a plant and soil perspective. Here, we summarize information on phytate availability in soils and phytate-P acquisition by plants. In addition, agronomic approaches and biotechnological strategies to improve soil phytate-P utilization by plants are discussed, and questions that need further investigation are raised. The information helps to better improve phytate-P utilization by plants, thereby reducing P resource inputs and pollution risks to the wider environment.



KEYWORDS: *organic P, phytate and phytase, availability, transgenic plants, organic acids, Pteris vittata*

1. INTRODUCTION

Phosphorus (P) is an essential and nonrenewable resource critical for agricultural production. On one hand, worldwide P reserves are limited and becoming harder to extract;[1] on the other hand, P is often fixed strongly in soils, thereby becoming unavailable to plants.[2] Due to the limited availability of P in many soils, excess fertilizer is applied to ensure optimal plant growth and crop yield annually.[3] The excess P, together with its inefficient use by plants, leads to large accumulation of unavailable P in soils.[4] Organic P (P_o) is the dominant P fraction in many soils, typically accounting for ∼50%, but can be up to 95% of total P in some agricultural soils.[5] This is because inorganic P (P_i) in fertilized soils is often transformed to P_o through microbial and plant activities. This is particularly significant in systems with large carbon reserves such as pastures, while it is less in some overfertilized soils where microbial immobilization capacity is saturated.[6,7] As such, improving the acquisition of P_o by crops has attracted much attention to enhance agricultural production.[8] Still, to use the finite P resources efficiently, a better understanding of soil P_o availability and factors constraining its plant acquisition is necessary, which helps to improve agricultural production and environment quality.[9]

Phytate. In agricultural soils, P_o is mainly present as the highly-phosphorylated inositol phosphate (IP), which exists in six phosphorylation states with 1−6 phosphate groups (i.e., mono, bis, tris, tetrakis, pentakis, and hexakis; IP_{1-6}) (Figure

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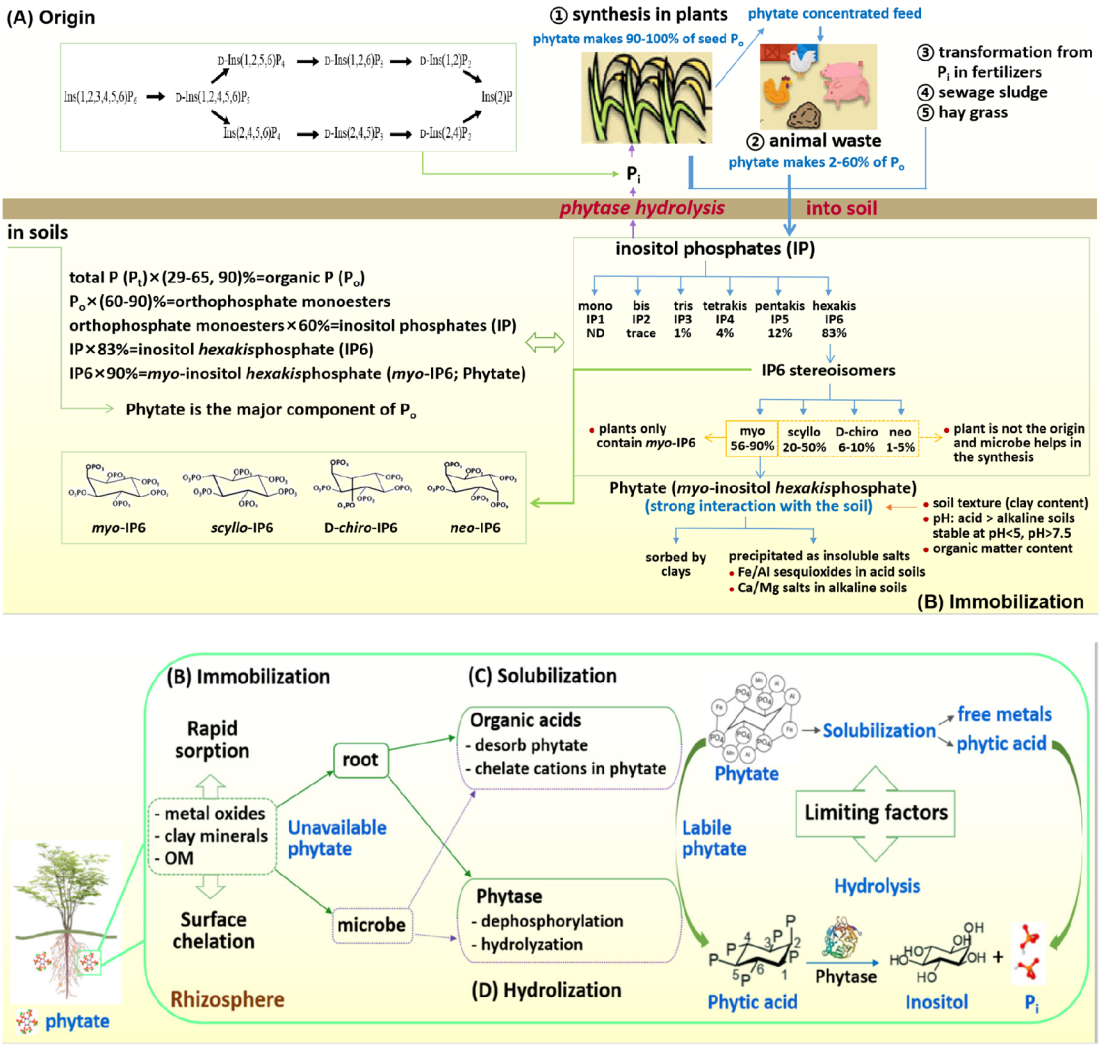

Figure 1. Conceptual model of phytate cycle in the environment: (A) origin from plants and animal wastes, (B) immobilization by sorption or chelation, (C) mobilization by exuding organic acids, and (D) hydrolyzation by plant- and/or microbial phytase.

1A).[10] In soils, IP is generally found as hexakisphosphate (IP_6; ~83% of IP). As its P is only completely stripped during dephosphorylation, it is rare to find other phosphorylation states (IP_{1-5}) in soils. The IP_6 occurs in soils in four isomeric forms (i.e., *myo*, D-*chiro*, *scyllo*, and *neo*) but predominantly occurs as the *myo* isomer (~56−90% of IP_6) with small amounts of other stereoisomers (20−50% of *scyllo*, 6−10% of D-*chiro*, and 1−5% of *neo*) (Figure 1B).[11,12]

Phytate (*myo*-IP_6), with six phosphate groups around its inositol ring, includes all metal derivatives of *myo*-inositol 1,2,3,4,5,6-hexakisphosphate.[4] Phytate is synthesized by plants to serve as the primary storage form of phosphate (up to ~90−100%) in plant seeds (Figure 1A). In soils, it can account for up to ~50% of P_o and ~80% of IP (Figure 1B), thus being an important source for plant P nutrition.[13]

Due to its six orthophosphate moieties, phytate is highly reactive in soils, with a molecular weight of 660 g mol^{-1} and 12 hydrogen donors in its structure.[4] With six phosphates on its inositol ring, phytate is not only bound to soils via sorption, surface complexation, and ternary phytate complexation (Figure 1B),[14] but also becomes incorporated into organic matter (OM) structures via Fe/Al bridges.[15] As such, large amounts of phytate can accumulate in soils and contribute to the soil P_o pool,[16,17] but with limited availability to plants.[18]

As the main P storage form in cereals and grains, phytate binds essential metal cations with low availability and is often introduced to soils via deposition of plant residues and manures from grain-fed animals, particularly monogastric animals, which lack phytase in their guts (Figure 1A). Phosphorus added to soils from the undigested phytate in animal manure poses potential pollution risks in areas of intensive animal production as it promotes eutrophication in aquatic systems, mostly surface waters like rivers, lakes, and oceans.[19,20] Despite the prevalence of phytate in soils, the understanding of its solubility and availability is inconsistent due to difficulties in its extraction, separation, and detection.

Phytase. Although phytate is important in maintaining P supply to crops, the mechanisms associated with its solubilization in soils and acquisition by plants are poorly understood.[21] As an essential macronutrient, P is taken up by plant roots as P_i.[22,23] As P_o, phytate must be hydrolyzed to release P_i into the soil solution before being taken up by plants.[24] Plants can secrete different phosphatase enzymes that target different P_o compounds, including phosphomonoesterase, phosphodiesterase, and phytase.[25−27]

Phytase (*myo*-inositol hexakisphosphate phosphohydrolase) is a class of phosphatase enzymes that specifically catalyze the hydrolysis of phytate to inositol, P_i, and free metals (Figure

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1C,D).[5] Phytate-P is released by phytase (EC 3.1.3.8, EC 3.1.3.72, and EC 3.1.3.26), often occurring at the 3, 5, and 6 phosphate positions. Phytase in the rhizosphere may originate from plant roots[28,29] and/or soil microbes[30] (Figure 1D). Their relative contributions to soil phytate hydrolysis are still unclear.[31]

To estimate phytate availability in soils, phytase-hydrolyzable P based on sequential extraction and enzyme hydrolysis has been used, which is the soluble P_o that can be hydrolyzed by phytase to be used by plants.[11,27] Soluble P_o can be obtained using various extractants including H_2O, $NaHCO_3$, NaOAc, citrate, NaOH-EDTA, and HCl, which account for different processes and represent different solubilities.[7,31,32] For example, water extracts estimate the P_o that might be transferred in runoff,[33] while $NaHCO_3$ extracts estimate the P_o that is readily mineralizable.[31] Citrate extracts estimate the P_o that is released by plant root exudates. This is because, among organic acids exuded by plants, citrate is the most abundant. Further, citrate shows greater extraction efficiency for P_o than bicarbonate such as $NaHCO_3$ (44−79% vs 1−9% of the P_o).[27,34,35] While NaOH-EDTA targets all phytate in soil as it can extract 71−90% of total soil P including phytate,[36] HCl extracts recover minimal phytase-labile P_o from soil.[31]

Crops that can utilize P_o in soils require less external P inputs, thereby reducing nutrient loss and consumption of nonrenewable mineral P.[37,38] Phytate can be hydrolyzed by phytase to enhance plant uptake, which is limited by the poor solubility of phytate and low activity of phytase in soils.[39] Therefore, enhancing phytate solubility and phytase activity is critical to improve phytate availability for sustainable use of P in soils.[40] Phytate can also become soluble after dissolution of OM that binds phytate.[24]

As such, this review aims to provide an overview of phytate availability in soils, especially the processes to improve phytate-P acquisition by plants. Understanding the mechanisms controlling phytate availability in soils helps to select plants/microbes that can exude organic acids/enzymes to enhance phytate-P utilization by crop plants.

2. PHYTATE IN SOILS

Phytate is stable in soils primarily due to its strong complexation by various metals and its strong binding to various components of soils.[6,17] This section covers its origin, abundance, forms, solubilization, and availability in soils.

2.1. Origin, Abundance, and Forms. *Origin.* Phosphorus accumulation as phytate in soils can reach up to ∼51 million metric tons annually, corresponding to ∼65% of the P fertilizer.[41] Soil phytate may come from plant tissues, monogastric animal manures, and microbial conversion from soil P_i. Phytate is synthesized by plants and microbes, with plants being the main source.[11,42] In particular, plants accumulate large amounts of phytate in the grains and seeds, being up to 80% of total P and 90−100% of P_o as a P reserve for seed germination (Figure 1A).[43] Phytate also occurs in other tissues but in smaller concentrations, which participates in molecular signaling and biochemical reactions.[44,45] In short, plant is an important source of soil phytate.[3]

Monogastric animals including poultry and swine cannot effectively utilize phytate-P in grain feed, and even ruminant animals like cattle and sheep are unable to mineralize all phytate-P, especially in high-phytate grain-based diets.[44] Phytate accumulation in animal manures is attributed to several factors, including its high concentration in grain-based diets, complexation with metals, and rapid passage through the digestive tract.[44] Therefore, phytate-rich animal manure is also an important source of phytate inputs to agricultural soils (Figure 1A).[46,47]

In addition, phytate can be transformed from immobilized P_i in soils (Figure 1A). George et al.[7] showed that P fertilization increased phytase-hydrolyzable P, attributing to the continuous accumulation of soil P. Even in soils after more than a decade without P fertilization, phytase-hydrolyzable P is still significantly greater than unfertilized soils.[7] The data indicate that phytate accumulation is associated not only with external inputs of phytate-rich substrates but also with soil immobilization and transformation from P fertilizers.[4]

Abundance. In soils, P_o is abundant and typically accounts for 40−95% of total P, with phytate being the major fraction, accounting for up to 50−80% of the P_o (Figure 1B).[5,18] Phytate concentrations in soil depend on land use and soil properties and vary with extraction methods. For example, phytate-P concentrations range from 1.4−220 mg kg^{-1} in arable soils to 42−220 mg kg^{-1} in crop and pasture soils and to 153−1325 mg kg^{-1} in manures (Tables 1A, 1B, and S1A). The average phytate-P concentrations are 457, 1047, and 2277 mg kg^{-1} in swine, cattle, and poultry manure, accounting for ∼16−17% of total P (Table S1B). The phytase-hydrolyzable P concentrations range from 0.1−0.4 mg kg^{-1} in water extracts to 26−189 and 153−613 mg kg^{-1} in NaOH-EDTA extracts of pasture soils and cattle dung in Southern Chile (Table 1A), averaging ∼20% of total P or ∼40% of P_o.[48−50]

The contribution of phytate to P_o also varies greatly among soils. For example, phytate-P concentrations in 47 Australian soils are 1−356 mg kg^{-1}, accounting for 0.4−38% of P_o. For Scottish soils and Chilean Andisols, they are 56−460 and ∼674 mg kg^{-1}, accounting for 24−58% and 42−67% of P_o (Table 1C).[51] In addition, phytate-rich animal manures (3413−8412 mg kg^{-1}) have often been used as fertilizers, thereby increasing soil phytate content. For example, after 10 years of applying swine manure to soils with conventional cultivation in Maine, soil phytate-P reached 118 mg kg^{-1} (Table 1A).[52] After 7 year of surface applications of 30 kg ha^{-1} dairy manure in Christiana soils with a permanent grass stand, soil plant-available P via Mehlich-3 is elevated by 78 mg kg^{-1}, with phytase-hydrolyzable P making up 48−55% of the extractable P.[53]

Forms. Of the six inositol phosphate esters (IP), i.e., mono-, bis-, tris-, tetrakis-, pentakis-, and hexakis-phosphates (IP_{1-6}), IP_6 is the predominant form, accounting for up to 83−100% of IP (Figure 1B).[17] There are also four stereoisomeric forms of IP_6, with the abundance being in the order of *myo* > *scyllo* > D-*chiro* > *neo*, representing 56−90, 20−50, 6−10, and 1−5% of IP_6 (Figure 1B).[11,12] Synthesized in plants, *myo*-IP_6 or phytate is the principal form and the most common IP in soils, with lower order esters being rare.[17] Since plants contain only the *myo* stereoisomer of IP_6, with chemical epimerization of *myo*-IP_6 being ruled out, microbes play a key role in synthesizing other IP_6 stereoisomers in soils (Figure 1B).[11]

Due to its higher degree of phosphorylation with six phosphate groups on its inositol ring, phytate has a high charge density, thereby interacting strongly with soils.[5] Phytate is bound to Fe/Al-oxides in acid soils and Ca/Mg minerals in alkaline soils.[15,19] For example, phytate sorption onto goethite and ferrihydrite is greater than that of P_i (3.8−12.7 vs 2.4−4.6 μmol m^2), and its binding to amorphous Al-oxide induces formation of stable Al-phytate precipitates (log K_{13-16} = 8.84−

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Table 1A. Classification and Amount of P and Phytate in Soils: Total P (P_t), Organic P (P_o), and Phytase-Hydrolyzable P (P_{Phy}) Concentrations (mg kg^{-1}) and Proportion (%) of the P_o to P_t and P_{Phy} to P_t or P_o in Different Soils by Different Extraction and Analytical Methods[a]

location	soil description	phytase-hydrolyzable P	% total P	% organic P	extraction and analytical method	ref
England and Wales	temperate lowland permanent pasture with high clay (22−68%)	26−189		ND−26		Turner et al.[161]
Madagascar	rice humid tropical oxisols	ND−33		11−35, 22	NaOH-EDTA extraction, solution ^{31}P NMR spectroscopy	Turner[162]
Southern Chile	dairy cattle dung	153−613	9−14	44−73%		Fuentes[163]
	feces	1325				Toor et al.[164]
Australia	pasture soils (n = 5)	0.1−0.4		10.8−33.5	H_2O extraction, enzymatic hydrolysis	Turner et al.[11]

location	soil description	dune age (years B.P.)	P_t	P_o	total IP_6	% of P_o	*myo*-IP_6	% of IP_6	extraction and analytical method	ref
Haast, New Zealand	mineral soils along coastal dunes under lowland temperate rain forest	290	178	128	56.7	44.4	34.9	61.6	NaOH-EDTA extraction, solution ^{31}P NMR spectroscopy, and spectral deconvolution	Turner[165]
		392	196	140	65.8	46.9	42.3	64.3		
		517	178	122	48.6	40.0	26.3	54.1		
		787	210	139	49.9	36.0	24.9	49.9		
		1826	126	99.0	51.1	51.6	27.2	53.3		
		3384	115	86.4	40.0	46.3	19.9	49.7		
		3903	85	66.9	29.7	44.4	17.0	57.1		
		4422	97	70.0	36.2	51.7	19.5	54.0		
		6500	82	63.2	26.4	41.8	12.7	48.0		

location	soil description	P_t	P_o ($\%P_t$)	P_{phy}	$\%P_t$	$\%P_o$	extraction and analytical method	ref
western USA	semiarid arable soils (n = 11)	220−1210	1.7−22.8	1.4−8.4		37−87	$NaHCO_3$ extraction, enzymatic hydrolysis, solution ^{31}P NMR spectroscopy	Turner et al.[166]
England and Wales	lowland permanent pasture soils (n = 29)	376−1981	208−895	26−189		11−35		Turner et al.[161]
Gooding County in southern Idaho	loamy fine sand + solid manure	440−970	71−170 (16−17)		10.2−16.2	4.8−16.2	NaOH-EDTA extraction, solution ^{31}P NMR spectroscopy	Hansen et al.[167]
	loamy fine sand + lagoon manure	750−1000	55−130 (7.3−13)		4.8−15			
Newport, ME (NS)	uncultivated soil (coarse-loamy, mixed, frigid, typic Haplorthod; 42% sand, 52% silt, and 6% clay)	12	2.2 (H_2O), 73 ($NaHCO_3$), 239 (NaOH)	0.7 (H_2O), 64.2 ($NaHCO_3$), 44.7 (NaOH)		20−71	sequential H_2O, $NaHCO_3$, and NaOH extraction, enzymatic hydrolysis	He et al.[159]
Presque Isle, Maine	conventional cultivation practice (caribou sandy loam: fine-loamy, isotic, frigid Typic Haplorthods; 51% sand, 41% silt, and 8% clay)	32.5	4.3 (H_2O), 90 ($NaHCO_3$), 249 (NaOH)	1.9 (H_2O), 83.3 ($NaHCO_3$), 54.0 (NaOH)		28−79		
	conventional cultivation practice with 10 yr swine manure application	37	6.8 (H_2O), 101 ($NaHCO_3$), 330 (NaOH)	1.0 (H_2O), 42.3 ($NaHCO_3$), 68.4 (NaOH)		17−49		
New Zealand	pasture soils (n = 24)	116−2746	46−991 (24−60)	13−220		14−91	NaOH-EDTA extraction, solution ^{31}P NMR	McDowell et al.[168]
Madagascar	rice Oxisols (n = 13)	130−1380	22−393 (19−44)	trace−33.1		12.2−26		Turner[162]

location	soil description	P_t	P_o ($\%P_t$)	P_{phy}	$\%P_t$	$\%P_o$	extraction and analytical method	ref
northern Alabama	fine loam (n = 1)	9.4 (H_2O), 9.1 ($NaHCO_3$), 67.1 (NaOH), 2.1 (HCl)	100 (H_2O), 25.2 ($NaHCO_3$), 45.4 (NaOH), 60 (HCl)		9.7−16.4 (NaOH)		NaOH extraction, with or without enzymatic hydrolysis, solution ^{31}P NMR	He et al.[169]

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Table 1A. continued

location	soil description	P_t	P_o (%P_t)	P_{inv}	%P_i	%P_o	extraction and analytical method	ref
	fine loam + poultry litter for 20 yr at rates of 1.36 mg ha^{-1} yr^{-1} (n = 1)	32.2 (H_2O), 82.2 ($NaHCO_3$), 230 (NaOH), 34.6 (HCl)	12.3 (H_2O), 0 ($NaHCO_3$), 37.6 (NaOH), 0 (HCl)		0.9–5.2 (NaOH)			
	poultry litter (PL)	9988–12436	6103–7700 (61–62)	5135–5968	48–51	77.5–84.1	NaOH-EDTA extraction, solution ^{31}P NMR	Hill and Cade-Menun[170]
Delmarva Peninsula	composted litter (CL): poultry litter and cow manure	11372–16256	3550–11090 (31–68)	3413–8412	21–52	75.9–96.1		
	crop soil (CS): soy or corn	326–827	95–141 (17–29)	42–53	6–13	37.6–44.2		
	ditch sediment (DS)	49–687	14.2–86.7 (10–29)	3–21	2–6	21.1–24.2		
Pennsylvania	fine loam (n = 10)	604–858	178–378 (28.2–60.9)	31–111	4.93–16.9	17.4–40.1		Dou et al.[171]
	fine loam + animal manure or spent mushroom compost (n = 10)	808–4866	114–244 (6.91–19.4)	53–106	1.57–6.76	22.6–43.4		
Irish	nonbasaltic grassland soils (n = 4)	616–2580	188–592	97–185		20–52		Murphy et al.[172]

[a]Numbers with underlines are the mean values.

20.1; Table 2B).[14,54] Besides minerals, phytate also binds strongly to OM.[55]

2.2. Sorption, Complexation, and Stability. Although phytate may be present in the soil solution, its direct uptake by plants has not been demonstrated.[18,45] Thus, to contribute to plant P nutrition, soil phytate must first be dephosphorylated from phosphate ester (C−O−P), phosphoanhydride (P−O−P), or phosphonate (C−P) via phytase-mediated hydrolysis.[54] However, it can only occur in the soil solution. Thus, its desorption from the solid phase is a prerequisite for its enzymatic hydrolysis by phytase. This section addresses its sorption, complexation, and stability in soils.

Sorption. Among P_o compounds, phytate has the strongest affinity for soils, whose immobilization and fixation are stronger than IP_{1-5} and P_i.[56] Its immobilization involves rapid sorption via surface complexation, which includes formation of phytate complexes with soil minerals[14] and incorporation of phytate into OM structures via Fe/Al bridges.[15]

Since phytate has 12 ionizable protons, with pK_a values being 1.1−12 for pK_1−pK_{12} (Table 2A), phytate is a strong ligand due to its high anionic charge at −6 to −10 under pH 4−10.[11] The six orthophosphate moieties and 12 replaceable protons in the phytate structure render its polyanionic property and strong ability to sorb onto soil solid phases and chelate with metal cations.[19] Phytate sorbs to metal oxides, clay minerals, and OM, with sorption capacity being ∼4 times that of P_i.[54] Depending on pH, phytate chelates metal cations to form sparingly-soluble precipitates, with Fe/Al complexes under acidic conditions and Ca/Mg complexes under alkaline conditions (Table 2B).[57]

Phytate sorption occurs through its phosphate groups, which react with metal oxides via ligand exchange through surface H_2O and OH groups, forming inner-sphere complexes.[58] Strong sorption of phytate has been demonstrated with calcite,[59] Illite, kaolinite, and montmorillonite,[60] goethite,[61,62] hematite,[63] ferrihydrite,[64] aluminum hydroxides,[65] and gibbsite,[66] especially at low pHs, as phytate sorption decreased with increasing pH. For example, phytate sorption on goethite and hematite decreased from 94% to 47% or from 0.95 to 0.38 μmol m^{-2} with pH increasing from 3 to 10.[62,63] Similarly, phytate sorption on ferrihydrite decreased by 25−61% with pH increasing from 5 to 9, with P1,3 and P2 phosphate functional groups showing preferential affinities at pH 5 and 8.5.[64] Moreover, the mechanism for phytate sorption is via formation of amorphous Fe-phytate precipitates on ferrihydrite surfaces.[67] However, phytate sorption onto gibbsite increases (0.47−0.52 μmol m^{-2}) with increasing temperature (4−55 °C) at pH 6, while it decreases (0.41−0.33 μmol m^{-2}) at pH 10 as the temperature is raised.[66] Phytate sorption onto soil minerals increases its negative charge, making it more reactive.[68]

Complexation. Complexation with metal cations occurs by ligand exchange and/or surface complexation, by which OH_2 or OH groups are replaced by the PO_4 anion.[15] Complexation can occur via one phosphate group, between two phosphate groups of a molecule, or between phosphate groups of different phytate molecules.[19] Phytate complexation with Fe^{3+} is stronger than Ca^{2+}, so Fe-phytate is more stable than Ca-phytate, with their stability constants (log K_{13-15}) at 8.89−18.2 and 8.3−8.4 (Table 2B). As such, Ca-phytate can be transformed to Fe-phytate in soils over time.[69] Besides, phytate incorporation increases the stability of Fe oxyhydroxide via

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Table 1B. Classification and Amount of P and Phytate in Soils: In the Whole Soil, NaOH-EDTA Extracts, and Bicarbonate Extracts of the 18 Western USA Soils and Phytase-hydrolyzable P (P_{Phy}) Concentrations in Bicarbonate Extracts of 11 Western USA Soils[d]

		whole soil[a]		NaOH-EDTA extractable P[b]		bicarbonate extractable P[b]				
soil origin	location and soil description	P	P_o (%)	P (%)	P_o (%)	P (%)	P_o (%)	P_{Phy} (%)	phytase used to determine phytate-P	ref
Western USA semiarid arable soils	Taunton	568	18 (3)	103 (18)	21 (118)	14.0 (2.5)	1.7 (9.8)	1.4 ± 0.32 (81)	phytase: *myo*-inositol hexakisphosphate 3-phosphohydrolase	Turner et al.[166]
	Warden	1210	67 (6)	175 (14)	63 (94)	19.3 (1.6)	3.7 (5.6)	1.9 ± 0.42 (50)		
	Amarillo	251	68 (27)	112 (45)	42 (62)	33.7 (13.4)	4.1 (6.0)	2.7 ± 0.26 (66)		
	Greenleaf	1058	88 (8)	205 (19)	63 (72)	17.7 (1.7)	4.3 (4.9)	2.3 ± 0.37 (53)		
	Portneuf (manured subsoil)	1135	172 (15)	255 (22)	55 (32)	110.5 (9.7)	4.7 (2.8)	3.8 ± 2.57 (81)	source: *Aspergillus ficuum*	
	Portneuf (manured)	1070	158 (15)	286 (27)	92 (58)	57.7 (5.4)	3.3 (2.1)	2.9 ± 0.28 (87)		
	Millville	762	189 (25)	224 (29)	89 (47)	11.9 (1.6)	5.4 (2.9)	2.6 ± 0.26 (48)	specified activity: 3.5 U mg^{-1} solid	
	Brinegar	626	130 (21)	214 (34)	91 (71)	40.2 (6.4)	15.0 (11.6)	6.5 ± 0.96 (44)	buffer: 2 M glycine-HCl, pH 2.5	
	Palouse	1000	189 (19)	284 (28)	144(76)	53.3 (5.3)	22.8 (12.0)	8.4 ± 0.39 (37)		
	Labenzo	1000	280 (28)	323 (32)	178 (63)	38.4 (3.8)	11.0 (3.9)	6.9 ± 0.83 (62)		
	Wahpeton	657	235 (36)	272 (41)	165 (70)	31.9 (4.9)	9.8 (4.2)	4.6 ± 0.86 (47)		
	Olton	220	44 (20)	73 (33)	29 (66)	19.1 (8.7)	1.8 (4.1)			
	Declo	827	119 (14)	221 (27)	90 (75)	21.7 (2.6)	2.5 (2.1)			
	Portneuf (conv[c] subsoil)	970	147 (15)	116 (12)	34 (23)	32.3 (3.3)	2.2 (1.5)			
	Williams	439	119 (27)	128 (29)	74 (62)	19.4 (4.4)	4.3 (3.6)			
	Portneuf (conv)	966	193 (20)	217 (22)	79 (41)	22.2 (2.3)	3.7 (1.9)			
	Roza	729	91 (12)	154 (21)	61 (67)	27.6 (3.8)	5.3 (5.8)			
	Portneuf (native)	890	189 (21)	192 (22)	68 (36)	39.2 (4.4)	7.8 (4.1)			

[a]Values in parentheses are % of soil total P_o to total P (P_t). [b]Values in parentheses are % of respective extractable P fraction to its concentration in whole soil. [c]conv − conventionally managed soils, P_t − total P, P_o − organic P, P_{Phy} − phytase-hydrolyzable P or phytate-P. [d]Values are means ± standard deviation of triplicate extracts. Values in parentheses are the proportion (%) of the P_t or P_o.

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Table 1C. Classification and Amount of P and Phytate in Soils: P_t, P_o, Inositol-P (INP), Humic-P (HA-P), Fulvic-P (FA-P), and Specific P Fraction/P_o Ratios in 15 Cultivated and Uncultivated (Native Grasslands) Chilean Volcanic Soils and 9 Representative Volcanic Soils under Grasslands[a]

soil type	soil description and no.	P_t	P_o (% P_t)	INP (% P_o)	HA-P (% P_o)	FA-P (% P_o)	extraction method	ref
Chilean volcanic soils	cultivated (+P)	1422−4011, 2582	870−3197 (42−80), 1618 (56)		(59−95, 61)	(5−41, 39)	hypobromide oxidation (Anderson, 1964)	Borie and Rubio[51]
	uncultivated (-P, native grasslands)	1150−3243, 1854	650−2375 (48−79), 1147 (62)		(43−81, 53)	(19−57, 47)		
Chilean volcanic soils under grasslands	Typic Distrandept 1	2348	1007 (43)	499 (49)	637 (63)	370 (37)		
	2	1925	1052 (55)	705 (67)	638 (61)	414 (c)		
	3	2697	1302 (49)	612 (47)	867 (66)	435 (33)		
	4	2327	1492 (64)	987 (66)	1041 (68)	478 (32)		
	5	2476	1450 (59)	612 (42)	965 (66)	485 (33)		
	6	3121	1310 (42)	750 (57)	841 (64)	469 (36)		
	7	2362	1208 (51)	778 (64)	793 (65)	415 (34)		
	mean	1925−3121, 2465	1007−1492 (42−64)	499−987 (42−67)	793−1041 (61−68)	370−485 (32−49)		
	Typic Vitrandept 8	1849	1083 (59)	709 (65)	721 (66)	362 (33)		
	9	1107	654 (59)	415 (63)	333 (51)	321 (49)		

[a]Values in parentheses are % of P_t or P_o; numbers with underlines are the mean values.

Table 2A. Protonation Constants of Phytate in Different Media and Ionic Strengths[b]

medium	ionic strength(mol L^{-1})	log K_1[a]	log K_2[a]	log K_3	log K_4	log K_5	log K_6	log K_7	log K_8	log K_9	log K_{10}	log K_{11}	log K_{12}
$(C_2H_5)_4NI$		*16.7*	*14.4*	12.2	9.92	7.53	6.11	3.53					
$(n\text{-}C_4H_9)_4NBr$		>12	>12	>12	11.5	7.97	6.41	3.93	2.73	2	<1.5	<1.5	<1.5
LiCl		9.71	9.46	8.63	7.6	6.27	5	2.63					
$NaNO_3$	0.1	9.48	9.98	9.53	8.2	6.49	5.17	3.02					
NaCl		9.58	9.84	9.5	8.14	6.5	5.25	2.88					
KCl		10.2	9.5	9.93	8.37	6.62	5.35	2.93					
CsCl		10.4	10.3	10.1	8.62	6.53	5.16	3.18					
$(CH_3)_4NCl$	0.15	10.8	10.5	10.3	8.79	6.9	5.72	3.1	1.9	1.9			
$NaClO_4$	0.15	8.59	10.5	9.02	7.82	6.13	4.88	2.49	1.98				
$(C_2H_5)_4NClO_4$	0.17	>13	>13	12.3	9.92	7.42	6.13	3.59	2	2.4	1	<1	<1
KCl	0.2	9.53	9.53	9.19	7.98	6.25	5.2	3.16	2.38	2.38	1.92	1.92	1.92
$(C_2H_5)_4NI$		*14.9*	*13.3*	11.6	9.79	7.5	6.12	3.61					
LiCl		9.06	8.81	7.96	6.93	5.63	4.39	2.08					
$NaNO_3$	0.5	8.73	9.39	8.82	7.57	5.88	4.59	2.6					
NaCl		8.93	9.19	8.83	7.48	5.88	4.65	2.37					
KCl		9.59	8.85	9.26	7.71	6.01	4.77	2.43					
CsCl		9.79	9.54	9.51	7.93	5.78	4.51	2.49					
$(C_2H_5)_4NI$		*13.6*	*12.5*	11.1	9.71	7.5	6.16	3.72					
LiCl		8.83	8.57	7.69	6.67	5.4	4.15	1.92					
$NaNO_3$		8.36	9.22	8.51	7.34	5.66	4.39	2.52					
NaCl	1	8.69	8.95	8.56	7.21	5.65	4.42	2.22					
$NaClO_4$		8.41	9.19	8.29	7.03	5.38	4.14	1.77	1.8				
KCl		9.35	8.61	8.99	7.45	5.77	4.54	2.28					
CsCl		9.82	9.38	9.41	7.77	5.57	4.34	2.33					
LiCl		8.6	8.34	7.34	6.35	5.18	3.95	2					
NaCl	3	8.47	8.71	8.21	6.89	5.43	4.22	2.3					
$NaClO_4$		8.29	8.62	8.01	6.61	5.07	3.86	1.52	1.63				
KCl		9.13	8.38	8.64	7.13	5.56	4.34	2.36					
NaCl	5	8.5	8.74	8.12	6.83	5.47	4.27	2.63					

[a]Predicated values in italics. [b]Adapted from Crea et al.[79]

inhibiting its transformation. For example, 10 months of aging at 22 °C or 60 h of hydrothermal treatment at 70 °C fails to transform the phytate-coprecipitated ferrihydrite (~60% is Fe-phytate) into hematite or goethite.[70] The data indicate that the strong complexation of phytate suppresses Fe polymerization and crystallization.[70] In the presence of Ca, phytate can form soluble complexes (Ca_1- or Ca_2-phytate) or insoluble precipitates (Ca_3-phytate) at all pH values.[54] Higher reactivity of phytate than P_i and other P_o compounds suggests that phytate undergoes strong immobilization, limiting it from being hydrolyzed by phytase, resulting in its low availability and high accumulation in soils.[71]

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Table 2B. Stability Constants of Phytate-Metal Complexes[b]

cation	ionic strength(mol L^{-1})	medium	t (°C)	log K_{13}	log K_{14}	log K_{15}	log K_{16}	other i:j species[a]
Mg^{2+}	0		10	7.93	6.49	5.47		2:3, 2:4, 2:5, 3:2, 3:3, 3:4, 3:5
	0		25	7.82	6.66	6.03		
	0.15	$NaClO_4$	37	10.5	9.76	8.76	7.25	1:2
Ca^{2+}	0		10	7.67	6.34	5.31		2:3, 2:4, 2:5, 3:2, 3:3, 3:4, 3:5
	0		25	7.64	5.82	5.41		
	0.15	$NaClO_4$	37		8.3	8.4	7.4	
Cd^{2+}	0.15	$NaClO_4$	37	9.7	8.76	7.53	6.92	1:2
	0.15	NaCl	25		5.25	4.71	4.42	1:7, 2:4, 2:5, 2:6, 2:7, 3:4
Cu^{2+}	0		25		10.3	7.79		2:5
	0.15		37	13.5	12.2	9.07	5.73	
Zn^{2+}	0.15	$NaClO_4$	37	11.3	10.3	8.54	6.94	
Ni^{2+}	0.15		37		8.78	8.44	7.20	
	0.10	KCl	36	7.27	5.96	5.18	5.05	1:0, 1:1, 1:2, 1:7
Co^{2+}	0.15	$NaClO_4$	37	9.1	7.9	6.96	6.26	1:2, 1:7
Hg^{2+}	0.15	NaCl	25	15.6	15.9	16.3	16.5	1:0, 1:1, 1:2, 1:7, 2:0, 2:1, 2:2
	0		25	14.7	15.1	15.5	15.7	
Mn^{2+}	0.15		37		8.78	8.44	7.2	
Fe^{2+}	0.15	$NaClO_4$	37	10.5	8.99	7.71	5.94	1:2
Fe^{3+}	0.15		37	18.2	12.7	8.89		1:2
Al^{3+}	0.15		37	20.1	16.4	12.2	8.48	1:2
$(CH_3)_2Sn^{2+}$	0		25	14.0	11.6	9.16	6.59	1:0, 1:1, 1:2, 1:7, 2:0−2:5, 3:0−3:5
$(CH_3)_3Sn^{+}$	0.05		25			2.45		2:5, 3:4, 3:5, 4:6, 5:1
	0.075		25			3.25		

[a] K_{ij} refers to the equilibrium: $iM^{n+} + H_jPhy^{(12-j)-} = M_iH_jPhy^{(12-in-j)-}$. [b] Adapted from Crea et al.[79]

Besides soil minerals and metal cations, phytate also binds to OM via Fe/Al-bridges. Coupled with Fe/Al, its sorption capacity exceeds 1.3 mM phytate-P mM^{-1} Fe/Al.[72] However, without Fe/Al, OM shows limited binding capacity for phytate, similar to P_i.[73] The data indicate that Fe/Al helps OM to sorb phytate (Figure 1B). Further, extraction with 1 M NaOH fails to liberate phytate from OM,[74] as it takes hydrolysis with 6 M HCl at 100 °C to release phytate from OM.[75] The data indicate incorporation of phytate into the Fe/Al-OM complex. As such, phytate bound to the Fe/Al-OM complex behaves differently from those bound to OM or Fe/Al-oxides.[5]

Stability. Phytate stability in soils is controlled by many factors including OM, clay type, clay content, pH, and metal oxides.[4,61] For example, peat soils contain greater amounts of phytate than sandy soils due to their greater OM content.[76] Clay type affects phytate sorption strength as phytate is more strongly sorbed to Illite than kaolinite.[68] pH impacts phytate sorption by soils, with more being accumulated in acid soils than alkaline soils. For example, after 24 h of reaction at pH 4.5, 2.12 μmol m^{-2} phytate is sorbed by ferrihydrite.[77] However, the amount sorbed is reduced by half at pH 6.5.[54] This is because phytate can complex with Fe, Al, Ca, and/or Mg, which is pH-dependent, being stable at pH < 5 (sorbs to Fe/Al minerals) and > 7.5 (precipitates with Ca).[11,58]

Besides, phytate stability varies with metal oxides, especially amorphous Fe and Al.[78] For example, phytate is sorbed onto goethite via four of the 6-phosphate groups, with the remaining two being free.[68] This explains the 3:2 sorption ratio between phytate and P in soils.[54] The large number of phosphate groups involved in phytate sorption leads to its stability with goethite, even in the presence of citrate and bicarbonate.[61] Unlike goethite, phytate sorption onto ferrihydrite occurs via two phosphate groups, showing less stability than onto goethite, with its desorption increasing with increasing pH.[77] In addition, phytate stability is metal-dependent, with Al^{3+} > Fe^{3+} > Mg^{2+} > Fe^{2+} > Ca^{2+}.[79] Their corresponding stability constants (log K_{13-15}) are 12.2−20.1, 8.89−18.2, 8.76−10.5, 7.71−10.5, and 8.3−8.4 in the $NaClO_4$ solution at 37 °C (Table 2B). These complexes are soluble only at pH < 2, as they are insoluble at mid-range pH values as Fe_4/Al_4-phytate (pH = 5−7) or Ca_6/Mg_6-phytate (pH > 7.5) complexes.[54,80]

3. PHYTATE-P UTILIZATION BY PLANTS

Phytate plays two roles in plants: serving as a reserve for P, inositol and minerals, and controlling P homeostasis.[45] Phytate is only available to plants after its solubilization and hydrolysis via phytase, with the released P diffusing to rhizosphere solution.[7,81] However, phytate is strongly bound to soils, so the concentrations of soluble phytate-P in the soil solution are typically very low (4−14.3 μg L^{-1}).[82] Therefore, plants and their associated microbes have developed strategies to solubilize and/or hydrolyze phytate to increase its availability.

3.1. Phytate Solubilization by Organic Acids. The accumulation of phytate in soils compared to other P-esters is attributed to its strong affinity for soils. The availability of soil phytate is low, hindering its interaction with phytase, thereby reducing its enzymatic cleavage of phytate ester bonds and the mineralization of its inositol ring.[83] Desorption and solubilization are two ways to increase phytate access by phytase.[5] In soils, P can be desorbed or solubilized by protons, organic acids, and phenolic acids, with organic acids being the primary factor in solubilizing sparingly-available P (Tables 3A, 3B, and S2).[84,85]

Organic acids contain carboxylate groups that can mobilize phytate via three mechanisms. First, carboxylates can desorb P anions from soil through ligand exchange by replacing P with a carboxylate anion. Specifically, tribasic citrate releases more P than dibasic oxalate due to its greater number of carboxyl groups, with closer pK_2 value (4.76 vs 4.28) to soil pH (4.5−9.5), leading to rapid degradation of oxalate.[86] Second,

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Table 3A. Summary of Known Plant to Mobilize Soil P

plant family/species	location and soil P concn (mg kg^{-1})				% total carboxylates			soil mobilized P[a] (mg kg^{-1})			
Fabaceae	location	P_t	bicarb.-extr.[b] P	total carboxylates (μmol g^{-1} root dw)[c]	malonic	malic	citric	malonic	malic	citric	ref
chickpea (*Cicer arietinum*)	Mullewa	83–97	17–19	40–65	70–79	7–20	8–12	1.6[d]	1.25[d]	2.0[d]	
	Merredin	82–108	11–24	100–310	63–82	10–19	7–22				
	Esperance	133–275	24–54	17–120	61–84	8–23	3–18				Wouterlood et al.[91]
Cicer arietinum Heera	Northam	158	5	30–70	50–91	30–41	trace	0.4	0.75	0.1	
Tyson	Nyabing	66	4		90–99	20–42		1.6	1.9	1.2	
white lupin (*Lupinus albus*)	Bindoon	–	–	237	–	88.5	11.5	–	–	–	
	Merredin			213		12	88				
	Pingrup			282		41.9	58.1				Veneklaas et al.[35]
	Mingenew			180		85.9	14.1				
	Nyabing			109		66.7	33.3				
	Scadden			92.8		29.4	70.6				

plant species	total carboxylates concn (μmol g^{-1} root dw)		organic acid species and % total carboxylates						pH		ref
			malic		citric		malonic		initial pH 6.7		
	3 μM P	300 μM P	3 μM P	300 μM P	3 μM P	300 μM P	3 μM P	300 μM P	3 μM P	300 μM P	
Triticum aestivum	5.00	3.33	90	93	10	6.31			4.04	4.09	
Brassica napus	3.33	8.00	95.4	98.7	2.8	–			4.35	4.70	
Vicia faba	6.66	5.00	62.8	68.4	38.5	32.3			6.52	6.78	
Lens culinaris	5.00	5.16	–	–	12.3	55.4	87.7	44.3	6.44	6.57	
Cicer arietinum	55.0	30.0	3.1	4.8	40	27.8	56.9	65.8	6.26	6.09	
Pisum sativum	25.0	9.3	4.8	34.9	95.4	63.1			6.26	6.37	Pearse et al.[173]
Lupinus luteus	51.6	18.0	10.8	13.8	90.7	87.7			4.70	5.17	
L. albus	49.1	20.0	38.8	44.8	58.6	55.7			5.22	5.39	
L. atlanticus	31.7	9.0	20.3	36.0	78.6	63.4			5.57	6.04	
L. angustifolius	28.3	14.2	28.5	36.9	72.7	67.1			5.57	5.70	
L. mutabilis	23.3	11.7	30.5	44.6	70.4	56.3			5.83	5.78	
L. pilosus	19.7	15.0	12.3	15.1	89.2	85.5		0.5	4.26	4.70	
L. cosentinii	16.7	14.7	12.3	13.8	88.4	86.3			5.22	5.39	

plant species	location and soil P concn (mg kg^{-1})			total organic acids (μmol g^{-1} root dw)			cultivars and % total organic acids									ref
							Sona			Kaniva			Tyson			
Fabaceae	location	P_t	bicarb.-extr.[b] P	Sona	Kaniva	Tyson	malonic	malic	citric	malonic	malic	citric	malonic	malic	citric	
chickpea (*Cicer arietinum*)	Bindoon	193	11.3	110	200	191	83	1	7	86.4	4.5	9.1	81	4.8	14.3	Veneklaas et al.[35]
	Merredin	57	6.0	11	36.4	45.5	54	<1	39	75	–	25	60	–	40	
	Pingrup	67	6.3	59	127	138	76	<1	22	64.3	7.1	28.6	67.1	4.6	28.3	
	Mingenew	116	9.0	37	168	228	67	2	28	97.3	–	2.7	84	12	4	
	Northam	113	6.0	120	–	–	63	–	37			–				
	Nyabing	40	4.3	100	144	167	80	–	20	87.3	6.3	6.3	87	6.5	6.5	
	Hyden	43	2.7	15	–	–	82	–	17			–				
	Scadden	18	5.3	122	133	150	81	4	15	65.8	4.8	29.5	63.6		36.4	

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Table 3A. continued

plant species		location and soil P concn (mg kg^{-1})			total organic acids (μmol g^{-1} root dw)			cultivars and % total organic acids									
								Sona			Kaniva			Tyson			
Fabaceae		location	P_t	bicarb.-extr.[b] P	Sona	Kaniva	Tyson	malonic	malic	citric	malonic	malic	citric	malonic	malic	citric	ref
		Mullewa	94	8.7	162	–	–	80	3	17				–			
		Morowa	91	10.3	52	–	–	78	3	19				–			
Proteaceae	organic acid species	concn (mg L^{-1} in 100 mL soil leachates			% total organic acids			ref									
Banksia integrifolia	citric			12.6			50										
	maleic			2.88			11										
	malic			4.58			18			Grierson[174]							
	aconitic			4.34			17										
	fumaric			1			4										

[a]The P-mobilizing capacity of carboxylates on soils was analyzed by extracting 3 g soil with 30 mL of 0.5 mM citrate, malate, or malonate. [b]bicarb.-extr. = bicarbonate-extractable; extr. = extractable. Bicarbonate-extractable P is extracted with 0.5 M sodium bicarbonate at pH 8.5.[89] [c]Carboxylates in the rhizosphere were extracted with 0.2 mM $CaCl_2$.[91,123] [d]Mobilized P was extracted from a soil with total and bicarbonate-extractable P at 66 and 4 mg kg^{-1}.[91]

carboxylates can solubilize Fe and Al via H^+, thereby destroying P sorption sites. Third, they can solubilize OM that binds to P via Fe/Al-bridges, with P being solubilized as the OM-Fe/Al-P complex.[15]

Since the interaction of phytate-P with soils is like P_i, similar reactions may solubilize phytate-P in soils but with more mechanisms being involved: 1) chelation of metals bound in metal-phytate complexes to release P and 2) chelation of metals to form complexes, which sorb to soils to prevent microbial degradation of organic acids, resulting in their long-lasting effect to improve phytate solubilization in soils.[24] Organic acids in the soil solution can be quickly degraded by microbes, whereas sorption onto soil hinders their degradation. For example, 70% of citrate added to soil is degraded after 10 d, but its sorption onto Fe/Al-hydroxide reduces its degradation by 50−90%.[87] The presence of organic acids in the soil solution is necessary for phytate solubilization in soils,[84,88] and the effects of organic acids on phytate-P acquisition by plants were summarized by Gerke.[24]

Phytate solubilization is essential for phytate-P acquisition by plants. Under P deficiency conditions, plant roots alter soil chemistry by releasing organic acids (Tables 3A and S2).[85] The typical organic acids exuded by plants include citrate, oxalate, malonate, gluconate, and acetate. In the rhizosphere, phytate solubilization and hydrolysis, and the subsequent P acquisition by plants are greater than bulk soil due to its greater organic acid concentrations.[84] The concentrations of organic acids in the bulk soil solution are generally <50 μM, but they can be in the range of 92.8−282, 15−50, and 45.4−228 μmol g^{-1} root dw in white lupin and chickpea, with citrate (63−88%) and malonic (60−81%) being predominant (Table 3A).[35,89]

Many plants exude organic acids, with those being effective including rape, chickpea, and lupin.[35] For example, cluster-forming plant species, such as white lupin and yellow lupin, excrete citrate to enhance P uptake under P deficiency.[90] In addition, organic acids such as citrate from legumes and malate from chickpea can solubilize phytate-P in soils, showing greater phytate-P acquisition compared to plants with limited exudates such as sunflower or wheat.[57,91,92]

However, plants like pea and chickpea are unable to access phytate in sand culture despite their ability to release organic acids into the rhizosphere.[93] Similarly, organic acids in the rhizosphere can not induce a significant difference in P acquisition from insoluble P by white lupin, implying that there is no simple relation between exudation of organic acids and available P in soil. It is possible that plant roots exude a basal level of organic acids into the rhizosphere. Plants increase the exudation of organic acids considerably when soil solution P availability is limiting (<1−2 μM), which often occurs in soils with a strong ability to bind P or nutrient-poor soil with sparingly-available P as Fe/Al-phosphate.[91,94] Therefore, further work is needed to establish the relationship between the concentrations of organic acids in the rhizosphere and the amount of phytate-P that can be taken up by plants in different soils.

3.2. Phytate Hydrolysis by Phytase. *Phytate Hydrolysis.* Phytate hydrolysis is mediated by phytase, which is classified according to its catalytic mechanism as belonging to histidine acid phosphatase (HAP), purple acid phosphatase (PAP), Cys phosphatase, or β-propeller phosphatase,[95] with HAP and PAP being more prevalent. Each group consists of several phosphatases, but only a few of them have phytase activity.[96]

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Table 3B. Summary of Microbially-Secreted Organic Acids to Mobilize Soil P

microbe species			mobilized P		
strain	organic acid species	exptl conditions	mg L^{-1}	pH	ref
D 5/23 *Pantoea agglomerans*	succinate, hydroxyglutarate, adipate, lactate, ketogluconate	200 μg P mL^{-1} as $Ca_3(PO4)_2$, 28 °C 7 d	62.8	5.93	Deubel and Merbach[175]
PsIA12 *Pseudomonas fluorescens*	succinate, lactate, malate, ketogluconate, galacturonate, citrate		44.1	4.77	
CC 322 *Azospirillum* sp.	gluconate, succinate, 2-ketoglutarate, ketogluconate		83.4	6.19	
Mac 27 *Azotobacter chroococcum*	citrate, malate, fumarate, succinate, lactate		98.1	4.84	
Msx 9 *Azotobacter chroococcum*	citrate, fumarate, malate, lactate, succinate		65.9	5.82	
ER 3	fumarate, isocitrate, lactate, malonate		75.5	5.32	
ER 10	lactate, gluconate, malonate, citrate		36.2	5.72	
	citric	200 mg L^{-1} P as $Ca_3(PO4)_2$, 28 °C 7 d	236 mg g^{-1}		
	succinic		178		
	lactic		126		
	citric	2 g soil +100 mL 5 g L^{-1} carboxylic acid, pH 7, 24 h	250 mg kg^{-1}		
	oxalic		175		
	gluconic		50		
	succinic		25		
A. calcoaceticus YC-5a	oxalic, malic, lactic, tartaric	5 g L^{-1} $Ca_3(PO_4)_2$, 28 °C, 7 d	518 ± 17.3	3.92 ± 0.02	Ren et al.[176]
E. agglomerans KMC-7	oxalic, lactic, citric, succinic		435 ± 15.6	4.13 ± 0.01	

microbe species		
fungi	organic acid species	ref
Aspergillus flavus, A. candidus, A. niger, A. terreus, A. wentii, Fusarium oxysporum, Penicillium sp., *Trichoderma isridae, Ttrichoderma* sp.	lactic, maleic, malic, acetic, tartaric, citric, fumaric, gluconic	Akintokun et al.[177]
Penicillium oxalicum	malic, gluconic, oxalic	Shin et al.[178]
Aspergillus flavus, A. niger, P. canescens	oxalic, citric, gluconic, succinic	Maliha et al.[179]
Penicillium rugulosum	citric, gluconic	Reyes et al.[180]
A. niger	succinic	Vazquez et al.[181]
Penicicllium variabile	gluconic	Fenice et al.[182]
	oxalic, lactic, glycolic, citric, succinic, tartaric	Whitelaw[183]

HAPs originate mainly from plants and show specific activity toward phytate. Their catalytic hydrolysis is via a N-terminal RHGXRXP motif and a C-terminal HD motif position to form an active site.[95] Unlike HAPs, PAPs originate from both plants and microbes and can hydrolyze various P_o forms besides phytate.[97] They are metallohydrolases that bind two metal cations in the active center. One of the cations is usually Fe^{III}, while other metals can be Zn, Mn, or Fe^{II}, which are responsible for PAP's color.[98]

Phytase activity in soils is affected by soil pH and its sorption.[8] Phytases show optimal activity toward phytate at 2.5−8.0 (Tables 4A and 4B) and then decline with increasing pH; thus, normally it is higher in acidic soils than alkaline soils.[99] Besides, phytase activity is inhibited due to its sorption onto soil minerals such as montmorillonite.[100]

Plant Phytase. Plant phytase is associated with various cellular functions, including energy metabolism, nutrient transport, metabolic regulation, and protein activation.[101] However, it is the extracellular phytase released from the roots that is of particular importance for phytate hydrolysis in soils.[45] Plant extracellular phytase is induced under P deficiency conditions, which either remains associated with root cell walls or is released directly into the rhizosphere to catalyze phytate hydrolysis.[30,102] For example, by exuding phytase into the rhizosphere, 1.7 μg P g^{-1} d^{-1} is released via phytate hydrolysis, facilitating phytate-P utilization by wheat.[102] Similarly, the arsenic-hyperaccumulator *Pteris vittata* (Chinese brake fern) can grow in Murashige and Skoog media supplied with phytate as the sole source of P.[29,94] After 40 days of growth, *P. vittata* takes up similar amounts of P grown in media with phytate or P_i, with tissue P concentrations being 2351 and 2208 mg kg^{-1}. In comparison, other plants including angiosperms (*Lactuca sativa*, *Trifolium subterraneum*, and *Allium schoenoprasum*) and pteridophytes (*Pteris ensiformis* and *Thelypteris kunthii*) fail to grow with phytate as the sole source of P.[29] The authors show the phytase activity in *P. vittata* roots at 0.018 U mg^{-1} (3×10^{-4} μkat mg^{-1}). However, for most plants, they do not show phytase activity in the roots as most of the phytate is stored in the seeds.[43]

More recently, Sun et al.[45] identified a novel root-specific phytase *PvPHY1* from *P. vittata* via prokaryotic expression, which can hydrolyze phytate, showing activity analysis at 37 °C and pH 5.5. Unlike typical plants such as rice and *A. thaliana*, expression of *PvPHY1* in *P. vittata* roots is greater than the fronds, which is consistent with the 7-fold stronger phytase activity in the roots than the fronds at 19.2 and 2.9 μmol P g^{-1} protein min^{-1}. Besides, expressing *PvPHY1* in tobacco plants enhances its growth by 0.7−1.1 g $plant^{-1}$ and P concentration by 10−50% under low- and adequate-P conditions.[45] Further, *PvPHY1*-expressed tobacco shows 25−32% less intracellular

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Table 4A. Summary of Known Plant Phytases to Hydrolyze Mobilized Phytate

plant species and fraction	phytase activity: U[a] mg^{-1}	phytase activity: μKat mg^{-1}	pH optim	temp (°C)	temp optim (°C)	K_m (μM)	molecular wt (kDa)	ref
buttercup squash			4.8	48	–	–	67	Goel and Sharma[184]
scallion leaves	–	–	5.5	51	–	200	–	Phillippy[185]
sunflower			5.2	55	–	290	–	Agostini and Ida[186]
tomato roots			4.3	45	–	38	164	Li et al.[187]
	205	3.44	4.3		50	–	–	
Lilium longiflorum	0.066	0.001	8.0		55–60	7.2	88	Scott and Loewus[188]
maize roots	5.7	0.1	5.0		40		71	Hubel and Beck[189]
Typha latifolia pollen	–	–	8	–	–	17	–	Hara et al.[190]
rye	–	–	6.0		45	300	67	Greiner et al.[191]
spelt	262	4.38	6.0		45	400	68	Konietzny et al.[192]
scallion (*Allium fistulosum*)	500	8.35	5.5		51	200	72	Phillippy[185]
maize seedlings	–	–	4.8		55	117	76	Laboure et al.[193]

plant species	substrate	activity (EU[b]×10^{-5})	ref
cereals (wheat, pearl millet, sorghum), legumes (mung, moth, cluster bean), oil seed crops (groundnut, sesame, mustard)	P-deficient	29.3	Yadaf and Tarafdar[194]
	phytin[c] (250 mg L^{-1})	29.1	
wheat (*Triticum aestivum*)	phytin (mg L^{-1})		
	50	2.32	
	100	3.87	
	150	5.25	
	200	7.89	
	250	9.35	
	300	9.82	
	500	9.87	

plant species	activity (EU[b]×10^{-5})	incubation condition	P released (mg L^{-1})	ref
sorghum (*Sorghum bicolor* SSG-1000)	65	5.4 × 10^{-5} EU, 500 mg kg^{-1} P as phytin, 2 wks	68	Tarafdar et al.[109]
cowpea (*Vigna unguiculata* RC-19)	69	10.8 × 10^{-5} EU, 500 mg kg^{-1} P as phytin, 7 d	136	
mung bean (*Phaseolus radiatus* K-851)	67			

plant species and fraction	phytase activity: P-fed plants	phytase activity: No-P plants	ref
wheat whole root extract (soluble; mU g^{-1} root fw)	4.4 ± 1.1	23.9 ± 1.2	Celi and Barberis[54]
wheat total intact root (mU g^{-1} root fw)		12.1 ± 4.0	
wheat external-root solution (mU g^{-1} root fw h^{-1})	<0.3	<0.3	

[a]One unit (U, μmol mg^{-1}) of phytase activity is the amount of phytase required to hydrolyze sodium phytate to produce 1 μmol P per min at 37 °C and pH 5.5.[203,204] [b]One EU corresponds to the amount of enzyme required to hydrolyze 1 μmol of *p*-nitrophenyl phosphate s^{-1} at pH 5.4, 35 °C.[194] [c]Phytin: Ca/Mg-phytate salts.

phytate and 30–56% more P in the roots, likely due to phytase-mediated hydrolysis of phytate within the roots.[45] However, *PvPHY1* expressed into tobacco plants fails to use phytate in the media, which is probably due to its inability to exude root phytase into the growth media. In comparison, *PvPHY1* in *P. vittata* roots can help to use extracellular phytate in the media. In short, *P. vittata* can grow on media with phytate as the sole source of P likely because it can exude phytase into the media to hydrolyze phytate for its uptake.

Though root extracellular phytase can help plants to obtain P from phytase-hydrolyzable phytate under sterile media,[103] phytase shows limited ability in soils.[104] This is because both phytase and phytate are readily sorbed by soils, with phytase activity being reduced by 95%.[104,105] This is consistent with wheat and pasture species growing in soils, which can use P from readily-hydrolyzable monoester (glucose-6-phosphate) and diester (ribonucleic acid) P_o substrates, but show limited capacity to acquire P from phytate (227–238 vs 74 μg P shoot^{-1}).[103] This is especially true in soils with high OM content and/or a history of P fertilizer applications.[11]

Microbial Phytase. Both plant roots and microbes possess phytase activity. However, the accumulation of phytate in soils indicates that phytate is resistant to mineralization compared to other P_o such as glucose 1-phosphate, nucleic acids, and phospholipids.[18] Phytate hydrolysis and subsequent plant P uptake have been assessed based on the depletion of phytase-hydrolyzable phytate in the rhizosphere.[106] However, there are conflicting results regarding the ability and relative contribution of root- and microbe-derived extracellular phytases to hydrolyze phytate in soils.[107,108]

For example, soil microbial phytase shows a greater ability to hydrolyze phytate than those from plant roots, i.e., 41.8–43.5 EU × 10^7 mL^{-1} filtrate for *Aspergillus niger* (*A. terreus* and *A. rugulosus*) vs 0.65–0.69 EU × 10^7 mL^{-1} filtrate for *Sorghum bicolor* (cowpea and mung bean) (Table 4B).[109] In addition, it is speculated that significant extracellular phytase activity from

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Table 4B. Summary of Microbially-Secreted Phytase to Hydrolyze Mobilized Phytate

microbe species	specific activity[a]		V_{max} (nKat mL^{-1})	residual activity after 24 h (nKat g^{-1} soil)				phytate-hydrolyzing capacity			ref
	U mg^{-1}	μKat mg^{-1}		soil type	initial activity	pH	solution phase	solid phase	soil type	incubation condition	P released (mg kg^{-1}soil)	
Aspergillus niger	282	4.7	0.112	spodosol	10.2	5.5	2.17	2.41	alfisol-1	water suspension (1:10 w:v), phytase (120 nKat g^{-1} soil), 24 h	0.05	George et al.[32]
				alfisol			0.96	3.62	alfisol-2		9.45	
				oxisol			0.24	0.72	spodosol		3.15	
				spodosol		7.5	9.40	0	vertisol		5.4	
				alfisol			0.84	0.24				
				oxisol			2.89	0				
Peniophora lycii	120	2	0.102	spodosol		5.5	9.40	0				
				alfisol			0.99	0.72				
				oxisol			8.68	0	alfisol-1		6.75	
				spodosol		7.5	8.19	0	alfisol-2		21.6	
				alfisol			0.87	0.24	spodosol		14.9	
				oxisol			8.44	0	vertisol		15.3	

microbe species	class of phytase	specific activity	pH optim	temp optim (°C)	pI[b]	K_m[c] (μM)	M_w[d] (kDa)	stability						ref
								pH	% activity	temp (°C)	half-life (min)	P (mM)	% activity	
Aspergillus ficuum			2.5							60	14.6	2	65.5	Kim et al.[195]
										70	0.5	4	42.0	
										80	0.2	6	12.6	
Bacillus amyloquefaciens DS11			7					4	80	70	1532	2	96.6	
								6	100	80	42	4	96.6	
								8	60	90	10	6	88.2	
								pH	% activity (Tris-HCl)	time (min)	% activity (95 °C)	Ca (mM)	% activity (95 °C)	
Bacillus subtilis 168		35 U mL^{-1}	6.6–7	55			44–47	5.5	96.6	15	43	1	47	
7.0								76.6	30	23	5	31		
		36.9 U mg^{-1}												Tye et al.[196]
8.5								3.33						
Bacillus licheniformis		28 U mL^{-1}	6.1–7	65				5.5	97	15	61	1	60	
		23.6 U mg^{-1}						7.0	86.6	30	40			
								pH	% activity (55 °C)	temp (°C)	% activity (pH 7)	metal (2 mM)	% activity (pH 7.5)	
Bacillus subtilis VTT E-68013	PhyC		7	55	6.5		43	5.5	28.6	37	58.5	Ca^{2+}	42	
												Zn^{2+}	15.6	
								7.0	85.7	55	85.7	Ni^{2+}	11.6	Kerovuo et al.[197]
												Mn^{2+}	10.7	
								8.5	11.4	75	15.7	Mg^{2+}	1.7	
												Cu^{2+}	1.4	

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Table 4B. continued

microbe species	class of phytase	specific activity	pH optim	temp optim (°C)	pI[b]	K_m[c] (μM)	M_w[d] (kDa)	stability: pH	stability: % activity	stability: temp (°C)	stability: half-life (min)	stability: P (mM)	stability: % activity	ref
			no $CaCl_2$					pH	% activity (no $CaCl_2$)	temp (°C)	% activity (no $CaCl_2$)	metal	% activity (no $CaCl_2$)	
Bacillus sp. KHU-10		36 U mg^{-1}	6.5–8.5	30–40	6.8	50	44	4.5	25	30	99.8	Cd^{2+}	0	Choi et al.[198]
								5.5	42.5	40	99.6	$Cr3^{+}$	45	
			in 10 mM $CaCl_2$					6.5	85	50	35	Cu^{2+}	59	
								7.5	100	60	0	Hg^{2+}	43	
			6.0–9.5	60				8.5	97.5			Mn^{2+}	17	
								9.5	77.5			Co^{2+}	60	

microbe species	class of phytase	specific activity	pH optim	temp optim (°C)	pI[b]	K_m[c] (μM)	M_w[d] (kDa)	stability: pH	stability: % activity (37 °C)	stability: temp (°C)	stability: % activity (pH 7)	stability: protease (0.1 g L^{-1})	stability: % activity (37 °C)	ref
Citrobacter braakii YH-15	P2	3457 U mg^{-1}	4	50		460	47	3	86	30	56	papain	85	Kim et al.[199]
								4	84	40	78			
								5	68	45	84		80	
		57.5 μKat mg^{-1}										elastase		
								6	56	50	100			
								7	46	55	68	pancreatin	70	
								8	13	60	34			
Escherichia coil ATCC 33965		1800 U mg^{-1}	4.5	60	6.3	540	44.7	2	46.3	30	33.2	pepsin (pH 2.5)	80	Golovan et al.[200]
								3	66.6	40	48.1			
								4	87	50	79.7	pancreatic proteases (pH 7)	38	
		30.1 μKat mg^{-1}						5	92.5	60	100			
					6.5	790		6	42.6	70	79.7	intestinal fluid	40	
								7	2.78	80	6.64			

microbe species	enzyme activity (EU × 10^{-7}): intracellular (g^{-1} fungal mat)	enzyme activity (EU × 10^{-7}): extracellular (mL^{-1} filtrate)	incubation condition	P released (mg L^{-1})	ref
A. niger	441 ± 38	43.5 ± 2.1	5.4 × 10^{-5} EU, 500 mg kg^{-1} P as phytin, 2 wks	185	Tarafdar et al.[109]
A. terreus	485 ± 17	44.7 ± 1.5	10.8 × 10^{-5} EU, 500 mg kg^{-1} P as phytin, 7 d	365	
A. rugulosus	433 ± 46	41.8 ± 2.2			

microbe species	specific activity[a]: U mg^{-1}	specific activity[a]: μKat mg^{-1}	pH optim	temp (°C): optim	temp (°C): maxim	pI[b]	K_m[c] (μM)	M_w[d] (kDa)	ref
Aspergillus niger	216	3.6	5.0	58	70	4.94–5.01	800	48.4–66.4	Ullah and Sethumadhavan[201] Lassen et al.[202] Vats and Banerjee[134]
Peniophora lycii	1374	22.9	5.5	58	70	3.61–4.37	800	44.6–72	

[a]Specific activity determined against phytate (*myo*-inositol hexakisphosphate). [b]pI: Isoelectric point. [c]K_m: Michaelis constant, indicating affinity. [d]M_w: Molecular weight.

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plants is rare, and soil phytase activity is mainly attributed to microbes. However, Belinque et al.[110] show that phytate-P acquisition by several plants is not improved with microbial inoculation, rather it is improved by plant-derived phytase. Besides, plant-derived phytase hydrolyzes phytate at a high rate, making acquisition of phytate-P similar to P_i.[110] This result is consistent with Tarafdar and Claassen,[111] suggesting that root phytase activity may be sufficient to hydrolyze phytate in the rhizosphere. On the other hand, microbial phytase may be important for microbial P turnover in soil.

The role of soil phytase in phytate hydrolysis was reviewed by Quiquampoix and Mousain,[112] but the role of microbes in phytate-P acquisition by plants is not well understood. The close relation between phytase activity in the rhizosphere and P acquisition by plants does not address the question whether the enzyme comes from plants or microbes.[106] Interestingly, recent research demonstrates that arbuscular mycorrhizal fungi show less ability to produce phytases than saprophytic fungi, but they compensate for this by recruiting hyphosphere bacteria that are able to produce phytase.[113] In fact, in some circumstances, these bacteria migrate to phytate hotspots along the fungal hyphae.[114] As such, the contribution of plant and microbial phytase in improving plant phytate-P acquisition needs further elucidation, especially in different soils.

Besides, phytase activity toward phytate is determined by both soil properties and microbial populations.[105] For example, in two soils with comparable pools of phytase-hydrolyzable phytate (12.5−17.0 mg P kg^{-1}), transgenic subterranean clover expressing phytase depletes ∼80% of phytate in a Spodosol soil with low ability for P retention, whereas only a small amount of phytate is depleted from an Alfisol with a greater P sorption capacity.[107] In addition, the fact that phytate depletion in soils is similar for all plants (control and transgenic) and unplanted controls indicates that the ability of a plant to obtain phytate-P is independent of plant species. Further, depending on soil type, it is more likely a function of microbial activity.[8] Nevertheless, this study highlights the potential contribution of phytate to plant P nutrition and the importance of microbial activity.

3.3. The Limiting Steps. Being the most abundant but also the most recalcitrant P_o in soils, phytate has the potential to contribute to plant P nutrition. Two hypotheses have been proposed regarding the limited acquisition of phytate-P by plants: 1) limited solubility of phytate due to its strong binding in soils and 2) low activity of phytase in soils makes phytate-P unavailable to plant roots.[5,24] As such, both phytate solubility and phytase activity are the limiting steps in plant acquisition of phytate-P.

It is generally known that soil phytate is relatively unavailable to plants, but findings are often inconsistent. In sand culture, Adams and Pate[115] show that both white lupin and narrow leaf lupin take up P_i and phytate-P at a similar rate, indicating little phytate sorption by sand and little limitation of P acquisition by a low phytase activity. Further, Lessl et al.[29] show that phytase from As-hyperaccumulator *P. vittata* roots can retain 93−98% of activity after being mixed with soils for a day, thereby helping phytate hydrolysis in the media and P utilization by *P. vittata*. Soil phytase is mostly effective in sand with low concentrations of organic matter, low microbial growth, and/or low sorption capacity.[115] The results agree with Tarafdar and Jungk[116] and Lung and Lim,[108] but are in contrast to Hayes et al.[18] and George et al.[107]

In P-fixing soil, both transgenic and nontransgenic lupin plants take up less phytate-P than P_i,[84] although phytate application increases plant P uptake. The results suggest that, in both plants, phytate-P acquisition is limited by phytate sorption onto soils, not phytase activity. Tarafdar and Claassen[117] and Lung and Lim[108] also conclude that phytate solubility is the limiting step in phytate-P acquisition by plants.

However, others show that phytase activity is the limiting step for phytate-P acquisition by plants.[40,118] Richardson et al.[103] find that wheat grown under sterile conditions with soluble phytate but not phytase activity is unable to use phytate as a P source. Similar results were reported for grasses and clovers.[18] Therefore, low plant phytase activity is a critical factor limiting phytate-P use under sterile conditions. On the other hand, some studies demonstrate phytate-P use by plants under nonsterile conditions, which may be attributed to microbial phytase in the rhizosphere.[119] This hypothesis is supported by increased plant P acquisition via microbial inoculation and microbial enzyme addition.[18,120,121] The results suggest that phytase activity on the root surface is the limiting step in phytate-P acquisition by plants, but this is only demonstrated in low-sorption capacity media such as agar.[107,122] Besides, the experiments fail to show the mechanism of how soil extracellular phytase improves phytate-P nutrition for plants.

As such, there is no agreement regarding the limiting step in phytate-P acquisition by plants. The possible reasons for the conflicting results may be due to the following: 1) phytate is often complexed with multivalent metals with low availability, whereas most experiments use sodium phytate with high availability; 2) variations among plant species with inherent phytase activity and therefore the ability to use phytate-P; 3) variations in the strength of phytate sorption in different soils, so that even plants with extracellular phytase cannot use phytate in all soils; and 4) substrates contain substances that may detach metals from phytate-metal complexes.

4. STRATEGIES TO IMPROVE PHYTATE-P ACQUISITION BY PLANTS

Factors affecting phytate availability, phytase activity, and phytate−phytase interaction determine the acquisition of phytate-P by plants. There are three main ways to help plants acquire phytate-P: 1) accelerating solution P depletion by plant uptake to increase phytate desorption from the rhizosphere; 2) improving phytate solubilization into the soil solution to increase its availability to phytase; and 3) increasing phytase activity to enhance phytate hydrolysis in the soil solution.

Plants can adapt to soils with limited available P via changing root features by forming longer root hairs and large roots, both increasing root surface area. This may be feasible only when soil solution P is not too low (>1−2 μM).[24] If soil solution P is too low, the diffusive flux of P to the root surface can not satisfy the P demand by plants. Under these conditions, plants and the associated microbes have developed strategies to increase rhizosphere P by secreting exudates (organic acids) and hydrolyzing enzymes (phytase).

4.1. Plant and Microbial Traits. *Plant Genotypes.* Organic acids and phytases exuded by plant roots vary across and within different species, which helps to select genotypes to improve phytate solubilization and hydrolysis.[93,94,123]

The most effective organic acids to solubilize phytate include those containing carboxylate groups, especially citrate and to a lesser extent oxalate,[24] which can exude 25−187 and 26−210

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μmol g^{-1} root dw (Table 3A). There is genetic variation across different plant species and intraspecific variation among different cultivars of a plant species. For example, white lupin from acidic and alkaline soils exhibits different root exudation and capacity to access Ca-phytate.[93] The composition and concentration of root exudates also vary among chickpea cultivars, with their concentrations in lateral roots increasing with plant growth.[91,123] Likewise, different abilities in plant root exudation are identified in pigeon pea cultivars.[124] In addition, cluster roots can help plants to efficiently uptake P by releasing organic acids. In a conventional single root, ~80−90% of its soluble P diffuses away, while the cluster roots can take up most of that soluble P.[24]

Phytases from different origins have different physicochemical and biochemical properties, which affect their mobility and ability to hydrolyze phytate in soils. Studies show the activities of extracellular phytase vary in different plants. For example, tobacco exudes phytase of the purple-acid-phosphatase class, which is responsible for Na-phytate utilization. The phytase shows a high affinity for Na-phytate (K_m = 14.7 μM) with specific activity at 6.03 μkat mg^{-1} and a V_{max} value at 7.2 μkat mg^{-1}.[125] George et al.[104] screened a range of wheat lines and identified considerable variation in extracellular phytase exudation among genotypes. Though relationships exist between root-exuded phytase activities and the ability to utilize phytate substrate in vitro, no clear relationships are demonstrated between extracellular phytase activities with P nutrition or plant growth when grown in soils.[126]

The data suggest that the variability in phytase activities among plants either has little effect on P nutrition of soil-grown plants or that the basal levels of phytase activities among plants are similar in their ability to hydrolyze phytate. However, it is more likely that the differences in plant-exuded phytase are masked by a much greater contribution of microbial-derived phytase.[105] Clarifying the capacity and condition of effective root exudation of organic acids and/or phytase benefits crop growth by increasing phytate solubilization.

Microbial Species. Root inoculation with microbes that produce organic acids helps to improve phytate solubility, thereby enhancing phytate-P acquisition by plants. Specifically, evidence shows that the symbioses of red clover -with arbuscular mycorrhizal fungi (AMF; *Glomus versiforme*) increase P solubilization in soils compared with nonmycorrhizal control plants, with AMF contributing 55−64% to shoot P uptake.[127] The data indicate that AMF hyphae play a main role in increasing soil P similar to the roots,[128] and it is critical to recruit phytate-solubilizing microbes to allow access to phytase in soils.[129]

Microbes that can secrete phytase have been identified via screening studies based on their abilities in utilizing phytate, homologue sequences, and protein databases.[4] The methodologies for screening phytase-producing microbes have been reviewed by Hill and Richardson,[130] which include both phytase positive and negative individuals. The methods for screening phytase-producing microbes (medium with phytate as the sole P source) in some cases select microbes that can solubilize (via organic acids) and/or hydrolyze (via phytase) phytate. The ability of isolated microbes in improving phytate availability has been identified. In one case, 39% *Pseudomonas* are negative for phytate utilization, but they become positive after citrate addition to the medium, suggesting these isolates can produce phytase to hydrolyze phytate, but their ability is hindered by limited phytate availability in soils.[130]

To improve phytate solubility, plant inoculants, e.g., *Pseudomonas* spp.,[131] *Citrobacter* sp.,[132] and *Pantoea* sp.[133] that can secrete organic acids into the rhizosphere have been found. For example, in vitro experiments show that Ca-phytate hydrolysis by phytase is improved in the presence of microbial organic acids, due to either Ca^{2+}-mediated phytase activation or solubilization via divalent metal chelation.[57,83] To increase phytate hydrolysis, plants are often inoculated with phytase-producing microbes. For example, pasture plants inoculated with phytase-producing *Pseudomonas* spp. increase their shoot P by 3.9-fold over control plants.[120] Recombinant *Pseudomonas fluorescens* CHA0 and *P. putida* KT2440 that overexpressed *Citrobacter braakii appA* (HAP-like phytase) improve phytate-P utilization of mung beans by 1.2−1.5-fold.[131]

Microbial phytases from different microbes are different in activity but are more abundant and with higher activities than plant phytases.[134] However, microbial phytase activity in soils has not been clearly linked to P nutrition. This is because microbes tend to secrete intracellular phytases, which do not play a role in extracellular phytate hydrolysis, instead being more related to cell metabolic functions. Despite this, phytase activity is often interpreted as an expression of microbial community metabolic requirements under P deficiency.[135] Besides, independent of the methodology, the environmental conditions and colony structure also affect the microbial ability to solubilize and hydrolyze phytate. For example, bioaggregates of microbes can improve P release from Al-phytate precipitates.[136]

At present, the understanding of the role of microbes in phytate solubilization and hydrolysis, and plant P nutrition is complex and incomprehensive.[119] Nevertheless, due to the large amount of phytate in soil and its potential contribution to plant P nutrition,[95] much research shows AMF's roles in improving soil phytate solubility. Specifically, they change the bacterial community structure and enhance phytate mineralization by carrying bacteria along their extraradical hyphae.[113,114] As such, biotechnologies using AMF's phytase enzymes to increase phytate bioavailability are desirable.

Plant Intercropping. Certain plants can be used in agriculture via intercropping to increase phytate availability by optimizing plants' contribution in modifying the soil P cycle. Their interactions in the rhizosphere are evident when plants with roots exuding phytase are intercropped with plants whose roots exude organic acids.[137] The benefit is greater with intimate interaction between phytase and organic acids when the roots are intermingled. For example, wheat when intercropped with white lupin shows improved phytate-P uptake and growth compared with a wheat monoculture, attributing to the ability of wheat roots to acquire more phytate-P, which is freed up by citrate from white lupin cluster roots.[138] Similarly, positive effects are apparent when wheat is intercropped with chickpea or pigeon pea is intercropped with rice or sorghum.[139]

Changes in Plant Root Traits. Several key morphological and physiological traits associated with P-uptake efficiency have been identified. In addition to plant and microbe strategies, agronomic practices can also improve phytate-P acquisition via facilitating root growth, enabling greater access to soil phytate, and ameliorating soil acidity and subsoil compaction. For example, breeding desirable root traits including rapid root growth, extensive root branching, and

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long dense root hairs are feasible by identifying specific genes.[105]

Though it is known that these morphological features can increase phytate availability in soils, there are few successful attempts to increase the efficiency of phytate-P use by crops.[105] This is largely due to the complexity of plant P-acquisition mechanisms and their responses to different environments. Further, the difficulty in identifying and selecting specific root traits in plant populations to increase P uptake, and compensatory effects of alternative mechanisms for a given environment make it difficult to implement.[140]

Despite these difficulties, it is possible to select enhanced specific P-acquisition processes such as selecting organic acids and/or phytase-producing genotypes to increase phytate utilization by plants or developing more phytate-efficient plants by manipulating desired traits through molecular biotechnologies.

4.2. Soil Management. Besides plant and microbial factors, phytate mobilization and mineralization are influenced by soil conditions, including pH, temperature, redox state, moisture, nutrients, and vegetation type.[141] Generally, mobilization is increased under anaerobic conditions and reduced with increasing labile P_i and organic C.[142] Mineralization is positively correlated with pH and temperature,[143,144] while its responses to the redox state and moisture are conflicted.[145]

Phytase shows the highest activity at optima pH, which ranges from 2.2 in yeast (*Pichia farinosa*) to 5.6 in *Rhizopus oligosporus* and 7.5 in *Bacillus subtilis* and mung bean (Table 4A).[112] However, pH optima can be changed within 1−2 units when phytase enters into soils. This is because at high pH, electrostatic interactions between the negatively-charged phytase and clay are repulsive. This prevents phytase adsorption, so it is free to diffuse into the soil solution and performs better activity.[112] Phytate mineralization is also affected by soil pH.[143,146] In 50 different British soils, phytate mineralization rates increase with soil pH from 3.9 to 7.1.[143] However, phytate mineralization only increases significantly as soil pH is at 6.5 compared to 5.0−6.0.[146] Moreover, phytate mineralization increases with exchangeable Ca concentration, indicating that soils developed from limestone parent material favor mineralization.[143] This can be attributed to the fact that Ca improves soil structure through aggregation and promotes microbial activity.[142]

Temperature affects phytate mineralization by influencing microbial growth and phytase activity.[142] Phytase activity peaks at 45−57 °C for *Bacillus subtilis*, while it decreases considerably at 80 °C and stops at 90 °C. Particularly, *Aspergillus fumigatus* and *A. niger* phytase are denatured at 50 °C (Table 4A, 4B).[112,147] However, phytase from As-hyperaccumulator *P. vittata* shows activity after being heated at 100 °C for 10 min, indicating its extreme heat-tolerance.[29] Normally, phytate mobilization and mineralization increase at temperature > 30 °C. Therefore, tropical forest soils with consistent temperature show greater mineralization than temperate forest, where phytate concentration tends to increase in winter and decrease in spring.[142]

The role of the redox state in phytate mineralization is complex, so the findings are inconsistent. Mineralization can occur under both aerobic and anaerobic conditions.[148] For example, Dick and Tabatabai[149] found greater mineralization under aerobic conditions, while Brannon and Sommers[145] reported higher mineralization under anaerobic conditions. The soil redox state affects phytate mineralization via affecting microbial populations, which are active in producing phytase.

Moisture is essential for phytase production and microbe survival. Phytase activity is positively correlated with soil moisture, and optimal hydrolysis of phytate is observed at 100% saturation.[142,150] For example, phytate mineralization is increased more during the wet season than the dry season; this is because moisture and nutrients stimulate microbial growth and their access to phytate.[151] Nevertheless, the correlation between flooding and mineralization is complex, so the significance of moisture in phytate mineralization remains uncertain.[142]

In short, many factors affect phytate mobilization and mineralization in soils. They are inter-related, making the outcome difficult to predict.

4.3. Genetic Engineering. Genetic engineering can be used for plants producing limited organic acids or extracellular phytases. Plants including subterranean clover, potato, *A. thaliana*, and tobacco-expressed microbial phytases can release extracellular phytase to utilize phytate-P.[107,131,152,153] For example, the transgenic expression of *Medicago truncatula* phytase gene (*MtPHY1*) in *A. thaliana* increases its root phytase activity by 12−16 fold, thereby increasing phytate-P acquisition and plant growth by 4.1−5.5 and 3.1−4.0 fold, respectively.[153] Besides, the secreted phytase and the associated gene have been characterized in the proteoid roots of white lupin.[154]

In addition to microbial phytases, plant phytase has been expressed in plants with limited phytase secretion. For example, expressing genes encoding extracellular phytase from Indian mustard into *A. thaliana* improves its phytase expression and secretion from lateral roots.[155] However, tobacco after expressing *P. vittata* phytase *PvPHY1* shows different results. Though tobacco P accumulation is increased by 10−50% and its growth is enhanced by 3.5−3.9 g plant^{-1}, tobacco plants fail to use phytate in the media.[45] The data indicate that, though phytase is probably exuded into growth media by *P. vittata*, thereby enabling its growth with phytate-P,[29] tobacco expressing *PvPHY1* fails to exude phytase into the media.[45] More research is needed to understand the controlling factors to make *PvPHY1* extracellular phytase.

Transgenic plants with extracellular phytase can hydrolyze phytate to enhance plant P nutrition and better growth under P-deprived conditions (Table S3)[4,152,156] and sand or sterile media.[29,107,131] For these experiments, plants are often grown in agar media using Na-phytate as a P source. For example, tobacco plants expressing *A. niger* phytase (*phyA*; *ex::phyA*) show increased extracellular phytase activity and accumulate 3.7-fold more phytate-P than control plants grown in sterile agar.[157] Moreover, the expressed phytase in tobacco from *B. subtilis* phytase (*168phyA*) has a higher K_m than the native enzyme, maintaining unchanged thermostability and catalytic activity at 2.3 U mg^{-1} protein (0.038 μkat mg^{-1}) in agar.[158]

However, compared to phytate in soils, which often binds to multivalent metals, Na-phytate is much more soluble, so the above results may not apply to soils. As such, when grown in soils, transgenic plants often show limited ability to access phytate-P. For example, the phytate-P utilization of transgenic tobacco overexpressing *A. niger* phytase (*phyA*) in soil conditions is similar to wild-type plants.[122,157] Even in soils with greater phytase-hydrolyzable phytate and greater extracellular phytase activity, subterranean clover does not show significant advantages in P nutrition and plant growth.[7]

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Further, tobacco grown in sand at pH 6 accumulates more P when supplemented with Mg-phytate than less-soluble Ca-phytate,[108] indicating the importance of phytate solubility during phytase hydrolysis. Therefore, although extracellular secretion of phytase is increased, poor availability of the phytate substrate due to its sorption by soil still constrains its activity in soils.

5. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Phosphorus is an essential nutrient for plant growth, and its management in soil is critical to ensure sustainable agriculture while protecting the environment.[1] Although soils often contain a large amount of P, only a small proportion is available to plants. For many soils, the use of P fertilizer and manure results in considerable P_o accumulation, especially in the form of phytate, which is relatively unavailable to plants. This review summarizes the following: 1) the origin, abundance, forms, solubility, and availability of phytate in soils; 2) limiting steps for phytate-P utilization by plants; and 3) strategies to improve phytate-P utilization by plants. The strategies include native traits to enhance the ability of plants and microbes to secrete organic acids and/or phytase. This can be achieved by selecting specific plant genotype or microbe species, plant intercropping, and genetic engineering. Genetic engineering can develop plants with increased phytase extracellular secretion by expressing microbial or plant phytase genes. Further, information regarding the limiting steps in phytate-P plant utilization, roles of OM-associated phytate, AMF, and manure phytase, and issues during practical application remains poorly understood and needs further study.

5.1. Limiting Steps in Phytate-P Acquisition by Plants. It is unclear whether soil phytate availability and/or phytase activity is the limiting step for phytate hydrolysis and its plant utilization, so efforts to understand the associated mechanisms are needed.

In terms of phytate availability, besides phytase-hydrolyzable phytate, there are other types of phytate in soils. Research shows that phytase-hydrolyzable phytate is not correlated with the growth or P acquisition of subterranean clover after expressing *A. niger* phytase *phyA*.[107,122] The limited P acquisition by plants in the presence of phytase indicates that water-soluble phytate not phytase-hydrolyzable phytate may be the phytate pool available to plants. However, water-soluble phytate is a smaller portion of phytase-hydrolyzable phytate in soils (0.7−1.9 vs 42.3−83.3 mg P kg^{-1}) (Table 1A) and animal manures (417 vs 708−1629 mg P kg^{-1}) (Table S1B).[50,159] As such, more attention should be paid to plant available phytate to clarify what constrains its access to phytase, thereby limiting its plant utilization. Besides, studies on extraction methods for available phytate and its predictability for plant-availability are needed.

In terms of phytase activity, besides increasing native phytase activity and genetic modification to increased phytase expression and extracellular secretion, the efficiency and performance of phytase once entering soils need more attention. Research shows that, with increased native phytase activity and transgenic-expressing extracellular phytase, soil phytate-P utilization is still limited,[32] suggesting reduced phytase activity in soils. Therefore, phytase catalytic adaptation to environmental conditions (e.g., soil texture, pH, temperature, and metal cations) to reduce inactivation, and the associated mechanisms need further investigations.

5.2. Further Efforts and Practical Issues. *OM-Associated Phytate.* Similar to orthophosphate, phytate binds not only to soil minerals but also to OM via Fe/Al bridges. As such, OM-metal-phytate complexes may be transported to the rhizosphere for hydrolysis and P uptake by plants.[17] However, experimental data on P availability of OM-associated phytate are old, so there is limited information on the interaction between phytate and OM.[74,75] More recently, Celi and Barberis[16] proposed hydrogen and covalent bonding as the mechanism, but this has not been tested.

Phytate Utilization in Manures. Given the worldwide scarcity in phosphate rock, a raw material for producing P fertilizer, it is necessary to utilize phytate in animal manures. During the production of animal feeds, phytase is added to facilitate phytate utilization. Since animal feeds are often rich in phytate, even with added phytase, manures with high phytate enter soils as amendments. As such, approaches to enhance the agronomic use of manure-derived phytate are needed. This way, manure phytate can be hydrolyzed to P before being applied to soil where it becomes poorly available. Another method is to increase phytate availability to plants. Coupling phytate reduction in manure and phytate uptake by plants helps to reduce P runoff and contamination of waters.

Plant and Microbial Processes. The organic acids and phytase in the rhizosphere arise from both plant roots and microbes, but their relative importance in contributing P acquisition is unclear.[160] Phytate utilization by plants is often based on experiments using sterile media, with results using nonsterile media being variable.[4,18] The continued discovery of widespread phytate-utilizing microbes and phytase-releasing plants may help to use recalcitrant phytate in soils.[29,45] Thus, contributions of phytase from plants and microbes and their efficiency in different environments need further research.

In addition, soil microbes are an integral component of the soil P cycle, so they play important roles in phytate transformation and hydrolysis. Still, the relative importance of microbial processes to use phytate and the interaction of different microbes (e.g., AMF and bacteria) with plant roots in facilitating phytate-P utilization need further elucidation.

Practical Application. From an application perspective, there are issues and challenges regarding these agronomic, plant, microbe, and molecular strategies to effectively utilize soil phytate.

For phytate solubilization mediated by organic acids, the challenge is whether it can be exploited to better intercept soluble phytate in competition with its fixation in soils. For phytase-mediated hydrolysis, when soils are limed to elevated pH, the benefit of phytase exudation may be reduced due to decreased phytase activity under alkaline conditions (optima at pH 2.5 and 5.0) and phytate precipitation with metal cations such as Ca and Mg. Besides, phytate and phytase are readily sorbed by soils, so the relationship between the concentrations of organic acids and activities of phytase with the amount of phytate-P that can be taken up by plants needs to be established. In this case, correlation indexes based on soil parameters, organic acid-dependent solubilization, phytase-dependent hydrolysis, and plant availability of different phytates can be incorporated into mathematical models to better evaluate phytate utilization potential by plants.

For agronomic practices to improve plant phytate-P acquisition, whether these options are practical for different

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agricultural systems remains to be determined. For genetic modification, transgenic plants need to be evaluated for their ability to access insoluble phytate in soils, which is often associated with metals and/or OM.

In short, plant- and microbe-based approaches have the potential to increase phytate-P utilization by plants. This is particularly relevant for organic farming where the use of soluble-P fertilizers is restricted by industry rules. Therefore, more research is needed for effective phytate-P acquisition by plants via developing plants that can secrete organic acids and/or synthesize phytase, which resist sorption to soils or retain activity when sorbed onto soils.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.2c00099.

Table S1, P_o fractions and 25 EDTA-extractable phytase-hydrolyzable P; Table S2, summary of known plant secreted organic acids to mobilize soil P; and Table S3, transgenic plant or yeast, phytase gene source, and expressed phytase activity and properties (PDF)

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Notes

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