Physiol Mol Biol Plants (April–June 2011) 17(2):93–103
DOI 10.1007/s12298-011-0062-x

REVIEW ARTICLE

Microbial phytases in phosphorus acquisition and plant growth promotion

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Published online: 7 May 2011

Abstract Phosphorus (P) is one of the major constituents in energy metabolism and biosynthesis of nucleic acids and cell membranes with an important role in regulation of a number of enzymes. Soil phosphorous is an important macronutrient for plant growth. Phosphorus deficiency in soil is a major problem for agricultural production. Total soil P occurs in either organic or in organic form. Phytic acid as phytate (salts of phytic acid) is the major form of organic phosphorus in soil and it is not readily available to plants as a source of phosphorus because it either forms a complex with cations or adsorbs to various soil components. Phosphate solubilizing microorganisms are ubiquitous in soils and could play an important role in supplying P to plants. Microorganisms utilizing phytate are found in cultivated soils as well as in wetland, grassland and forest soils. Various fungi and bacteria (including plant growth promoting rhizobacteria) hydrolyze this organic form of phosphorus secreting phosphatases such as phytases and acidic/alkaline phosphatases. A large number of transgenic plants have been developed which were able to utilize sodium phytate as sole source of phosphorus. However, the recombinant phytases were similar to their wild type counterparts in terms of their properties. Increased phytase/phosphatase activity in transgenic plants may be an effective approach to promote their phytate-phosphorus utilization. The extracellular phytase activity of transgenic plant roots is a significant factor in the utilization of phosphorus from phytate. Furthermore, this indicated that an opportunity exists for using gene technology to improve the ability of plants to utilize accumulated forms of soil organic phosphorus. This review is focused on the role of phytases and phytase producing microbes in promoting the growth of different plants.

Keywords Phytase · Phytate · Plant growth promoting rhizobacteria · Organic phosphorus · Plant growth promotion · Transgenics

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Introduction

Phosphorus is one of the major constituents in energy metabolism and biosynthesis of nucleic acids and cell membranes and it has an important role in regulation of a number of enzymes (Rodríguez and Fraga 1999; Richardson et al. 2009a, b). Being a critical macronutrient for plant growth and development, most part of the total soil phosphorus is unavailable for uptake due to rapid immobilization by soil organic and inorganic components (Rodríguez and Fraga 1999; Tarafdar and Gharu 2006; Jorquera et al. 2008). Phosphorus is limiting for crop production and it has been estimated that the world resources of inexpensive rock phosphate may be depleted by 2050 (Vance et al. 2003). The lack of inexpensive phosphorus has been recognized as a potential future crisis in agriculture (Abelson 1999). Improving the plant phosphorus uptake and its utilization will have some significant impacts on both agriculture and environment (Rodríguez and Fraga 1999; George et al. 2009; Wasaki et al. 2009; Richardson et al. 2009a, b).

Organic phosphorus which is about 30 to 80 % of soil phosphorus plays an important role in the phosphorus cycle

of agricultural soils (Dalal 1977; Tarafdar and Gharu 2006). The predominant form of organic phosphorus are phytates (inositol hexa- and penta-phosphates), which constitutes up to 60 % of soil organic phosphorus (Fig. 1). This organic form is poorly utilized by plants (Tarafdar and Junk 1987; Tarafdar and Claassen 1988; Rodríguez and Fraga 1999; Mudge et al. 2003). Solubilization of phytate is quite important for the mobilization of phytate-P for plant uptake (Tarafdar and Junk 1987; Tarafdar and Claassen 1988; Turner et al. 2002; George et al. 2005; Tarafdar and Marschner 2005; George et al. 2009; Wasaki et al. 2009). This must be dephosphorylated by phosphatases (phytases and phosphatases) [Fig. 2a-b], before assimilation by the plants (Tarafdar et al. 1988; Tarafdar and Rao 1996; Richardson 2001; Yadav and Tarafdar 2003; Yadav and Tarafdar 2007a, b; Richardson et al. 2009a, b).

In the rhizosphere, organic substances exuded from plant roots are utilized by microorganisms as readily available sources of carbon and energy for their growth and reproduction (Whipps 1990). However, rhizosphere microorganisms have to compete with plant roots for most other elements such as phosphorus. Phosphatase activity is high within the rhizosphere, which is responsible for the hydrolysis of organic phosphorus (Tarafdar and Junk 1987; Yadav and Tarafdar 2003; Yadav and Tarafdar 2007a, b). Phytase is one of the phosphatases responsible for the sequential hydrolysis of phytate to a series of *myo*-inositol phosphate derivatives and inorganic phosphate (Tarafdar and Claassen 1988; Rodríguez and Fraga 1999; Yadav and Tarafdar 2003; Yadav and Tarafdar 2007a, b).

Most of the plant species can not utilize this organic source of phosphorus due to the lack of adequate levels of extracellular phytase. However, when phytase was added exogenously (Hayes et al. 2000; Idriss et al. 2002; Singh and Satyanarayana 2010), or when the phytase gene from *A. niger* was expressed in transgenic plants (Richardson et al. 2001a), the plants were able to uptake phytate phosphorus. Therefore, the presence of phytase in the rhizosphere may enhance plant growth under field conditions (Singh and Satyanarayana 2010). Phytase activity derived from soil microorganisms such as *Sporotrichum thermophile* (Singh and Satyanarayana 2010), *Discosia* sp. FIHB 571 (Rahi et al. 2009), *Pseudomonas* sp. (Richardson et al. 2001b) and *Bacillus amyloliquefaciens* (Idriss et al. 2002) has been shown to contribute to plant growth promotion.

Plant-growth-promoting rhizobacteria (PGPR) are free-living bacteria in the rhizosphere that have beneficial effects on the growth of plants (Kloepper et al. 1988; Bloemberg and Lugtenberg 2001; Patel et al. 2010). The effect of PGPR on plant growth promotion has been attributed either due to the production of plant-growth regulating substances (Steenhoudt and Vanderleyden 2000) or due to the enhancement of nutrient availability (Nautiyal et al. 2000). Microorganisms utilizing phytate are found in cultivated soils as well as in wetland, grassland and forest soils (Richardson and Hadobas 1997). But the utilization of phytate by crops and microbes is generally limited under field conditions due to the formation of insoluble phytates and adsorption to soil particles (Hayes et al. 2000). This hypothesis is supported by the fact that phytase activity could be stimulated by application of organic acids (Singh and Satyanarayana 2010; Hens et al. 2003). The secretion of organic acids from plant roots enhances phosphorus availability by chelating cations such as Fe^{+2}, Al^{+3}, or Ca^{+2}, which form insoluble phytates and also help in the sulubilization of insoluble phytates (Tang et al. 2006; Singh and Satyanarayana 2010). Adams and Pate (1992) proved this hypothesis in white lupin (*Lupinus albus* L.) a high organic acid producer which showed more vigorous growth than other species with sodium phytate as the sole phosphorus source.

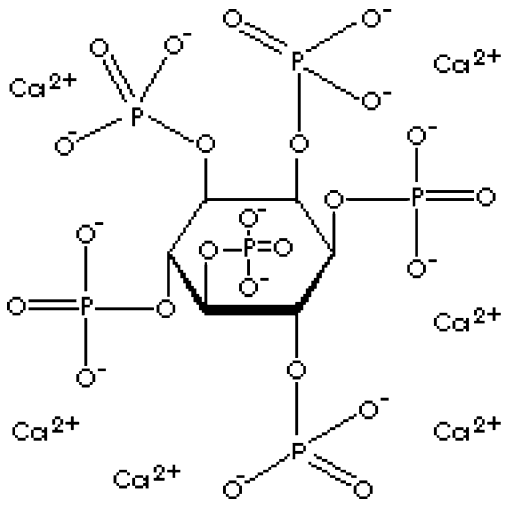

Fig. 1 Interaction of divalent metal cations with phytic acid

Soil phosphorus and its mechanisms of acquisition

Phosphorus is present at a level of 400–1,200 mg kg^{-1} of soil (Fernández and Novo 1988). The amount of soluble phosphorus in soil is usually very low (Goldstein 1994; Rodríguez and Fraga 1999). The biggest reserves of phosphorus are rocks and other deposits that provide a cheap source of phosphate fertilizers for crop production (Halder et al. 1990; Odum 1986). The phosphatic rock deposits in India are about 40 million tons (Roychoudhury and Kaushik 1989). Mineral forms of phosphorus present in the soil are mainly insoluble and they constitute the biggest reservoirs of this element in soil. Under suitable conditions, they can be solubilized and become available for plants and microorganisms (Fernández and Novo 1988; Rodríguez and Fraga 1999; Richardson et al. 2009a, b).

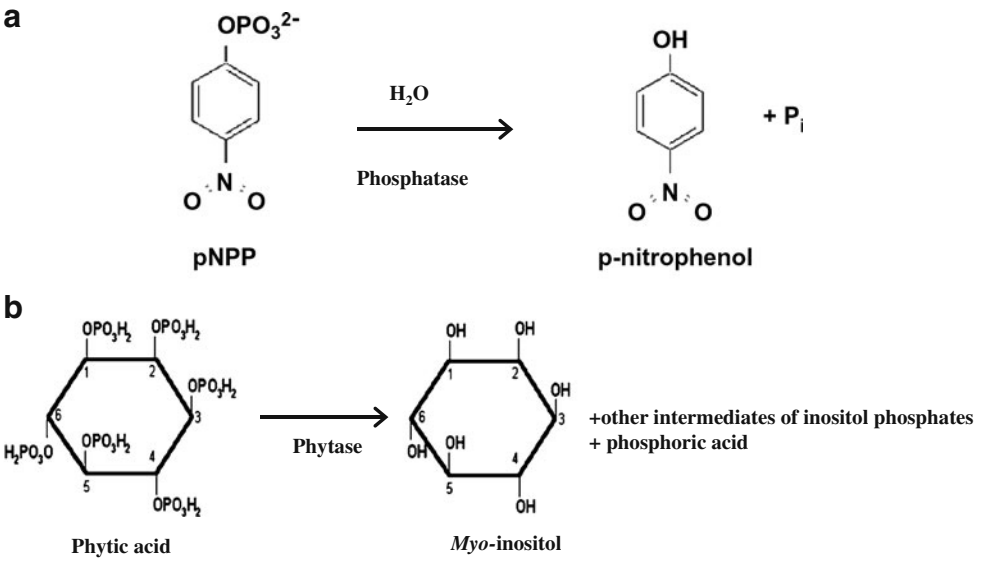

Fig. 2 **a**. Reaction catalyzed by phosphatase on p-nitrophenyl phosphate **b.** Reaction catalyzed by phytase on phytic acid

A considerable part of soil phosphorus in agricultural land is contributed by the applications of P fertilizers (Richardson 1994). Due to the rapid immobilization, a large portion of soluble inorganic phosphate becomes unavailable to plants after application (Dey 1988). The type of soil and its pH greatly influence the fixation and precipitation of phosphorus in soil. In acidic soils, phosphorus is fixed by free oxides and hydroxides of aluminum and iron, where as in alkaline soils it is fixed by calcium such as super calcium (Goldstein 1986; Jones et al. 1991). Superphosphate having sufficient amount of calcium precipitates half of its own phosphorus in the form of dicalcium phosphate (Lindsay 1979). The organic form of phosphorus is the second major component of soil phosphorus which constitutes 30–50 % of the total phosphorus in most soils (Paul and Clark 1988) and it is mostly present in the form of *myo*-inositol phosphate i.e. phytates (Singh and Satyanarayana 2010; Tang et al. 2006; Rodríguez and Fraga 1999; Anderson 1980; Harley and Smith 1983). Other organic phosphorus compounds beside phytate include phosphomonoesters, phosphodiesters including phospholipids and nucleic acids, and phosphotriesters (Paul and Clark 1988; Rodríguez and Fraga 1999).

Role of microbes in phosphate acquisition

Soil contains a wide range of organic substrates, which are source of phosphorus for the growth of plants. But it must be hydrolyzed to inorganic phosphorus before its assimilation by the plants. The hydrolysis of most of the organic phosphorous compounds is carried out by means of phosphatase enzymes. The presence of a significant amount of phosphatase/phytase activity in soil has been reported (Tarafdar et al. 1988; Lynch 1990; El-Sawah et al. 1993; Feller et al. 1994; Kremer 1994; Rodríguez and Fraga 1999). Microbial phosphatase activity has been detected in different types of soils (Tarafdar and Junk 1987; Kirchner et al. 1993; Garcia et al. 1992; Xu and Johnson 1995; Kucharski et al. 1996; Rodríguez and Fraga 1999; Richardson et al. 2009a, b). The presence of organic phosphorus hydrolyzing bacteria in soil has been studied by Greaves and Webley (1965) for the rhizosphere of pasture grasses, by Raghu and MacRae (1966) for rice plants, as well as by Bishop et al. (1994) and Abd-Alla (1994). Yadav and Tarafdar (2004) has studied the phytase activity in the rhizosphere crops, trees and grasses under arid conditions. of Fox and Comerford (1992) observed a significant acid phosphatase activity in the rhizosphere of slash pine in two forested Spodosoils, whereas, Burns (1983) studied the activity of various phosphatases in the rhizosphere of maize, barley, and wheat, showing that most of the phosphatase activity was observed in the inner rhizosphere where soil pH ranged from acidic to neutral. Soil microbes expressing a significant level of acid phosphatases/phytases include strains from the genus *Rhizobium* (Abd-Alla 1994), *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus, Klebsiella* (Thaller et al. 1995), *Pseudomonas* (Gügi et al. 1991; Richardson et al. 2001b; Ryu et al. 2005), *Bacillus* (Skrary and Cameron 1998), as well as *Sporotrichum thermophile* (Singh and Satyanarayana 2010), *Emericella rugulosa* (Yadav and Tarafdar 2003, 2007b), *Discosia* sp. FIHB 571 (Rahi et al. 2009) and some other fungi.

A large portion of soil and rhizosphere microorganisms is able to utilize phytate as carbon and phosphorus source (Greaves and Webley 1965; Richardson and Hadobas 1997; Rodríguez and Fraga 1999). These studies provide evidence that support the role of microbes in making organic phosphorus available to plants (Tarafdar and Claassen 1988). The extracellular phytases are important for the

degradation of phytate in the rhizosphere (Mudge et al. 2003; Yadav and Tarafdar 2007a, b; Patel et al. 2010; Singh and Satyanarayana 2010). Some examples of phytase and or phosphatase secreting soil bacteria and fungi capable of phosphorus release from different organic sources are shown in Table 1.

Organic phosphate occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus compounds. The saprophytic microbes decompose organic matter in soil, which release orthophosphate from the carbon structure of the molecule (McGrath et al. 1995). The microbial degradation of organic phosphorus is highly influenced by environmental factors as well as physicochemical and biochemical properties of the molecules (Paul and Clark 1988; Ohtake et al. 1996; McGrath et al. 1995, 1998). The hydrolysis of these compounds is carried out by means of the action of several phytases and other acidic/alkaline phosphatases (Fig. 2a-b). The phosphatases may be acidic or alkaline depending upon optimal pH for their activity. On the basis of substrate specificity they are classified as specific or nonspecific acid phosphatases (Cosgrove et al. 1970; Burns and Beacham 1986; Pradel and Boquet 1988; Rossolini et al. 1998; Rodríguez and Fraga 1999).

Phytase producing microbes and their effect on plant growth

The insoluble forms of phosphorus are transformed to an accessible soluble form by many soil microorganisms (Tarafdar et al. 1988; Tarafdar and Rao 1996; Richardson 2001; Yadav and Tarafdar 2003; Yadav and Tarafdar 2007a, b; George et al. 2009; Richardson et al. 2009a, b; Wasaki et al. 2009). A list of microorganisms showing growth promoting effect on plants is given in Table 2. These microbes are called as plant growth-promoting microorganisms (PGPM).

Organic phosphorus must be dephosphorylated by phosphatases before assimilation by the plants, because plants acquire phosphorus as inorganic phosphorus (Richardson 2001). The microbial population in the rhizosphere utilizes the organic substances exuded from plant roots for their growth and reproduction (Hiltner 1904; Whipps 1990). Therefore these microbes have to compete with plant roots for other elements such as phosphorus. Major part of soil organic phosphorus is phytates, mainly consisting of inositol penta- and hexaphosphates (Dalal 1977). Microbial population utilizes this P by secreting phosphatase and phytase in the rhizosphere (Tarafdar and

Table 1 Microorganisms and enzymes responsible for plant growth promotion

Enzyme type	Microbial strains	Reference
Acid phosphatase	*Pseudomonas* sp.	Richardson et al. 2001a, b
	P. fluorescens	Ryu et al. 2005
	Burkholderia cepacia	Unno et al. 2005
	Enterobacter aerogenes	Thaller *et al*. 1995
	E. cloacae	Thaller *et al*. 1995
	Citrobacter freundi	Thaller *et al*. 1995
	Proteus mirabali	Thaller *et al*. 1995
	Serratia marcenscens	Ryu *et al*. 2005, Hameeda *et al*. 2006
	Emericella rugulosa	Yadav and Tarafdar 2007a, b
	Chaetomium globosum	Tarafdar and Gharu 2006
Phytase		
	Bacillus sp.	Ryu et al. 2005
	B. subtilis	Ryu et al. 2005
	B. mucilaginosus	Li et al. 2007
	B. amyloliquefaciens	Idriss et al. 2002
	B. pumilus	Ryu et al. 2005
	B. circulans	Hameeda et al. 2006
	Pseudomonas putida	Richardson and Hadobas 1997
	P. mendocina	Richardson and Hadobas 1997
	Sporotrichum thermophile	Singh and Satyanarayana 2010
	Aspergillus niger	Hayes et al. 2000
	Discosia sp.	Rahi et al. 2009
	Emericella rugulosa	Yadav and Tarafdar 2007a, b
	Chaetomium globosum	Tarafdar and Gharu 2006

Table 2 Phytase sources and their effects on plants

Phytase source	Plant growth promotion	Reference
Burkholderia sp.	Lotus	Unno et al. 2005
Discosia sp.	Maize, Pea, Chickpea	Rahi et al. 2009
Bacillus sp.	Arabidopsis	Ryu et al. 2005
Pseudomonas fluorescens		
Serratia marcescens		
Rhizobacteria	Tomato	Hariprasad and Niranjana 2009
	Pigeon pea	Patel et al. 2010
Serratia marcescens Pseudomonas sp.	Pearl millet	Hameeda et al. 2006
Bacillus circulans		
Emericella rugulosa	Pearl millet	Yadav and Tarafdar 2003; 2007a, b
Chaetomium globosum	Wheat, Pearl mllet	Tarafdar and Gharu 2006
A. rugulosus	Wheat, Chick pea	Tarafdar and Rao 1996
Sporotrichum thermophile	Wheat	Singh and Satyanarayana 2010
A. niger	Sub Clover	Hayes et al. 2000
Bacillus subtilis	Tobacco, Arabidopsis	Lung et al. 2005
A. niger	Arabidopsis	Richardson et al. 2001a, b; Mudge et al. 2003
Medicago truncatula	Arabidopsis	Xiao et al. 2005
Bacillus mucilaginosus	Tobacco	Li et al. 2007

Junk 1987; Tarafdar et al. 1988; Tarafdar and Rao 1996; Richardson 2001; Yadav and Tarafdar 2003; Yadav and Tarafdar 2007a, b; George et al. 2009; Wasaki et al. 2009).

Unno et al. (2005) isolated over 300 phytate-utilizing bacterial strains from the rhizosphere of *Lupinus albus* (L.). These isolates were classified as *Burkholderia* based on 16S rDNA sequence analysis. Many bacterial isolates were able to utilize insoluble phytates. When co-cultured with *Lotus japonicus* seedlings, some isolates showed positive effect of plant growth promotion. A fungus, *Discosia* sp. FIHB 571 isolated from tea rhizosphere showed plant growth promoting effects due to the solubilization of inorganic phosphates, production of phytase and siderophores, and synthesis of auxins (Rahi et al. 2009). The fungal inoculum significantly increased the length of root and shoots and dry matter in maize, pea and chickpea over the control.

Plant growth promoting rhizobacteria

Plants roots are colonized by some bacteria called as plant growth-promoting rhizobacteria (PGPR). PGPR are well known to exert beneficial effects on plant growth and development. Ryu et al. (2005) studied the mechanisms by which PGPR stimulate plant growth promotion. Some PGPR strains such as, *Bacillus amyloliquefaciens* IN937a, *B. subtilis* GB03, *B. pumilus* T4, *B. pumilus* SE-34, *B. pasteurii* C9, *Paenibacillus polymyxa* E681, *Serratia marcescens* 90–166, and *Pseudomonas fluorescens* 89B-61, were studied for their effect on growth promotion of wild type and mutant Arabidopsis in vitro and in vivo. In vitro study revealed that all eight PGPR strains increased foliar fresh weight of Arabidopsis as compared to control plants. In vivo studies showed that all bacterial strains promoted foliar fresh weight under greenhouse conditions. Some plant hormone mutants of Arabidopsis were generated to study the effect of bacterial strains in signal transduction pathways both in vitro as well as in vivo. The stimulation of plant growth promotion by PGPR strains in vitro involved signaling of brassinosteroid, IAA, salicylic acid, gibberellins while in vivo ethylene signaling pathway was involved.

Jorquera et al. (2008) isolated various phytate-mineralizing bacteria (PMB) and phosphate-solubilizing bacteria (PSB) from the rhizosphere of perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*), wheat (*Triticum aestivum*), oat (*Avena sativa*), and yellow lupin (*Lupinus luteus*) growing in volcanic soil in Chile. Among 300 isolates, six phosphobacteria were selected, based on their ability to utilize both Na-phytate and Ca-phosphate on agar media. These selected phosphobacteria were genetically identified as strains of P*seudomonas*, *Enterobacter*, and *Pantoea*. All selected strains showed the production of phosphatases that resulted in higher inorganic phosphate liberation compared with uninoculated controls. Patel et al. (2010) observed significant increase in dry shoot/root ratio and P content of shoot in Na-phytate containing semi-solid agar inoculated with rhizobacterial isolates.

Among the 43 isolates from rhizospheric soil of tomato, 33 were found to be positive for solubilizing both inorganic and organic forms of phosphorous (Hariprasad and Niranjana

2009). On the basis of their ability to colonize roots of tomato and to increase the seed quality parameters 16 isolates were selected. All selected isolates showed improved shoot length, root length, fresh weight, dry weight and phosphorous content of tomato seedlings as compared to control under green house study. Analysis of rhizosphere soil samples of 30 day old-seedlings revealed that the available phosphorous content was high in rhizospheric soil samples of plants raised from seeds bacterized with these isolates over control. However, some isolates showed protection against *Fusarium* wilt only.

Plant growth-promoting bacteria (PGPB) have been reported to affect the growth, yield, and nutrient uptake by plants by different mechanisms. Among various isolates of PGPB from farm waste compost (FWC), rice straw compost (RSC), Gliricidia vermicompost (GVC), and macrofauna associated with FWC, seven isolates significantly increased shoot length and ten isolates showed significant increase in leaf area, root length density, and plant weight (Hameeda et al. 2006). Maximum increase in plant weight was observed by *Serratia marcescens* EB 67, *Pseudomonas* sp. CDB 35, and *Bacillus circulans* EB 35. All the three composts showed improved growth of pearl millet. Inoculation of composts with bacteria further improved plant growth up to 88 % by RSC with EB 67, 83 % with GVC and EB 67.

Yadav and Tarafdar (2007a) studied the effect of phytase and phosphatase producing actinimycetes on growth of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.). The organic phosphorus source was made available to the plant by the action of phytase and phosphatase of actinomycetes. A phosphatase and phytase producing fungus *Emericella rugulosa* was isolated and tested under field condition in a loamy sand soil (68 % organic phosphorous as phytate) using pearl millet as a test crop (Yadav and Tarafdar 2007b). The fungal contribution was significantly higher than the plant contribution in the hydrolysis of the different organic phosphorus compounds. The fungal inoculation resulted in significant improvement in plant biomass, root length, seed and straw yield and phosphorus concentration of root and shoot as compared to control plants.

An extracellular HAP-phytase producing thermophilic mould *Sporotrichum thermophile* promoted the growth of wheat seedlings (Singh and Satyanarayana 2010). The growth and inorganic phosphate content of the plants treated with fungus were better than the control plants. The compost prepared by the combined action of native microflora of wheat straw and *S. thermophile* promoted the growth of wheat seedlings (Singh and Satyanarayana 2010). The inorganic phosphate content of the wheat plants was also high as compared to those cultivated on the compost prepared either with only native microflora or *S. thermophile*. The difference in the plant growth promoting effect was distinguishable after 10 days, and it further became prominent after 30 days.

Phytase and plant growth promotion

The plant roots in the rhizosphere interact with different physical, chemical and biological properties of soil (Yadav and Tarafdar 2003; 2004; 2007a, b). Plant roots interact with soil microorganisms which have impact on plant nutrition either directly, by influencing nutrient availability and uptake, or indirectly through plant growth promotion. When supplied with glucose-1-phosphate, seedlings of the six species (three legumes and three grasses) grew as well as plants supplied with inorganic phosphate, where as phytate was a poor source of phosphorus for plant growth (Hayes et al. 2000). Using phytate as sole source of P for the growth of *Trifolium subterraneum*, it was found that the plant roots secreted a very low level of phytase. Addition of *A. niger* phytase in the medium liberated sufficient phosphorus available to enable *T. subterraneum* seedlings to grow as well as plants supplied with inorganic phosphorus. The efficiency of hydrolysis of different organic phosphorus compounds by different fungi indicated that the fungi have enough potential to exploit native organic phosphorus to benefit plant nutrition.

Phytase and phosphatases producing fungi were used as seed inoculants, to efficiently utilize phytate phosphorous in soil. (Yadav and Tarafdar 2003). A phosphatase and phytase producing fungus, *Chaetomium globosum* was tested as an inoculant for wheat and pearl millet crops (Tarafdar and Gharu 2006). A significant improvement in plant biomass, root length, plant P concentration, seed and straw yield and seed P content was observed after inoculation with the fungus. Among various *Aspergillus* species, *A. rugulosus* was found to be superior that resulted in improved dry matter and grain yield in wheat and chick pea due to acquisition of phosphorus (Tarafdar and Rao 1996). Tarafdar and Marschner (2005) studied the effect of co-inoculation of a phyase producing fungus (*A. fumigatus*) and a VAM fungus (*Glomus mosseae*) on wheat grown in two heat-sterilized low-phosphorus soils supplied with sodium phytate. Seed inoculation with the *A. fumigatus* or soil inoculation with *G. mosseae* resulted in increased shoot and root dry weight and root length, phosphatase activity in the rhizosphere and shoot concentrations of P and to a lesser extent of K and Mg. However, the results were better when both fungi were co-inoculated.

An extracellular HAP-phytase of a thermophilic mould *S. thermophile* promoted the growth of wheat seedlings (Singh and Satyanarayana 2010). The growth and inorganic phosphate content of the plants were better than the control. Among different concentrations of sodium phytate, 5 mg plant^{-1} was adequate for liberating enough phosphorus for

the growth of the seedlings. The plant growth, root/shoot length and inorganic phosphate content of test plants were better than the control plants. An enzyme dose of 20 U plant^{-1} was found adequate to liberate enough amount of inorganic phosphate required for supporting plant growth. The plant growth, root/shoot length and inorganic phosphate content of test plants were higher than the control (Singh and Satyanarayana 2010). A tobacco (*Nicotiana tabacum*) root PAP phytase secreted from roots under P starvation (Lung and Lim 2006) has been identified recently which is involved in mobilizing organic phosphorus in soil (Lung et al. 2008).

Heterologous expression of phytase in plants

Extensive use of chemical fertilizers to improve soil fertility and agricultural productivity has had a deleterious effect on the environment. Therefore, there is a need to reduce the use of these chemical fertilizers. To maintain the sustainability of agriculture, one approach is to improve the ability of crop plants to acquire P from organic sources (George et al. 2009).

Transgenic plants expressing microbial phytases have been proved as an innovative model for exploiting the organic P source present in the soil. Several experiments have been performed for expressing phytase gene in transgenic plants (Table 3). Transgenic plants might contain adequate levels of phytase to avoid the exogenous supplementation of feed and food with microbial phytases and or inorganic phosphate in order to meet their daily need (Rao et al. 2009; Mudge et al. 2003; Richardson et al. 2001a, b). For phytase production, transgenic plants could be used as bioreactors.

Transgenics with fungal phytases

Among fungal phytases, the most studied phytase (*A. niger* phytase) has successfully been expressed in tobacco, soybean, alfalfa, wheat, Arabidopsis, maize and canola (Pen et al. 1993; Verwoerd et al. 1995; Ullah et al. 1999; Li et al. 1997; Gutknecht 1997; Brinch-Pedersen et al. 2000; Richardson et al. 2001a, b; Mudge et al. 2003; Ponstein et al. 2002; Chen et al. 2008; Li et al. 2009). However, the properties of the recombinant phytases are similar to their wild type. The recombinant enzyme was secreted into the apoplast in tobacco which account for approximately 14 % of the total soluble protein (Verwoerd et al. 1995). The recombinant phytase expressed from tobacco leaves had the same temperature optima with less glycosylation and showed a moderate shift towards acidic pH (Ullah et al. 1999). The recombinant phytases expressed in soybean and alfalfa also had almost the same properties as the wild fungal phytase with difference in their glycosylation (Li et al. 1997; Ullah et al. 2002). The recombinant phytase which was expressed in tobacco released inorganic phosphate from animal feed under standard conditions. There was no loss of activity even after 1 year (Pen et al. 1993). The recombinant phytases produced in soybean and canola seeds showed the similar performance in feeding trials as microbial phytases (Denbow et al. 1998; Zhang et al. 2000). Gutknecht (1997) expressed phytase in the alfalfa plant and the transgenic plant was used as a bioreactor instead of animal feedstuff. The recombinant phytase was recovered in juice after the plant processing. *Aspergillus fumigatus* phytase was expressed in tobacco using *Agrobacterium* mediated transformation (Wang et al. 2007). The recombinant protein accumulated in leaves upto 2.3 % of

Table 3 Transgenic plants expressing phtytases

Phytase source	Expressed in	Reference
A. niger	Tobacco	Pen et al. 1993; Verwoerd et al. 1995; Ullah et al. 1999
	Soybean	Li et al. 1997
	Alfalfa	Gutknecht 1997
	Wheat	Brinch-Pedersen et al. 2000
	Arabidopsis	Richardson et al. 2001a, b; Mudge et al. 2003
	Canola	Ponstein et al. 2002
	Maize	Chen et al. 2008
A. fumigatus	Tobacco	Wang et al. 2007
Escherichia coli	Rice	Hong et al. 2004
Selenomonas ruminantium	Rice	Hong et al. 2004
Bacillus subtilis	Tobacco	Yip et al. 2003
B. subtilis	Tobacco, Arabidopsis	Lung et al. 2005
Aspergillus sp.	Maize	Drakakaki et al. 2005
Medicago truncatula	Arabidopsis	Xiao et al. 2005

total soluble protein and it was highly thermostable. A phytase gene from *A. niger* was expressed in soybean that resulted in improved growth and P acquisition by the transgenic plants (Li et al. 2009).

The transgenic Arabidopsis harbouring *A. niger* phytase gene plants secreted phytase only from roots when grown on medium under low phosphate conditions (Mudge et al. 2003). The transgene enabled the plants to grow on medium containing phytate as a sole source of phosphorus. The growth rates and shoot phosphorus concentrations of these plants were similar when grown on phytate or phosphate as the phosphorus source. The growth and phosphorus nutrition of *A. thaliana* plants supplied with phytate was improved significantly after the introduction of phytase gene from *Aspergillus niger* (Richardson et al. 2001a, b). Growth and phosphorus nutrition of the transformed plants was improved and was equivalent to control plants supplied with inorganic phosphate suggesting the extracellular phytase activity of plant roots as a significant factor in the utilization of phosphorus from phytate and opportunity for using gene technology to improve the ability of plants to utilize accumulated forms of soil organic phosphorus. Drakakaki et al. (2005) generated transgenic maize plants expressing *Aspergillus* phytase either alone or in combination with the iron-binding protein ferritin. There was 95 % degradation of endogenous phytic acid present in the seeds, with a concomitant increase in the amount of available phosphate.

Furthermore, phytase in the maize seeds resulted in increased cellular iron uptake and the rate of iron uptake was correlated with the level of phytase expression. The transgenic white clover lines expressing phytase gene were established from cotyledon using *Agrobacterium tumefaciens*-mediated transformation method (Shengfang et al. 2007). Using phytate as sole phosphorus source, the phytase activities in root of transgenics were higher as compared to control. The phosphorus concentration of plants, the accumulative phosphorus amount per plant, plant fresh weight, and plant dry weight were much higher in transgenic lines than in controls.

Transgenic plants with bacterial phytases

Like fungal phytases, bacterial phytase also have been expressed in transgenic plants. *Escherichia coli* and *Selenomonas ruminantium* phytases were expressed in germinated rice seeds. The phytase activity was 60 times more than the nontransformant, without any adverse effect on plant growth and development (Hong et al. 2004). The phytase gene from *B. subtilis* was also expressed in tobacco and Arabidopsis (Lung et al. 2005). In tobacco and Arabidopsis phytase activities in transgenic leaf and root extracts were seven to nine times higher than those in wild-type extracts; whereas the extracellular phytase activities of transgenic plants were enhanced by four to six times (Lung et al. 2005).

Li et al. (2007) showed that both wild type *Bacillus mucilaginosus* and transgenic (containing phytase gene) strains promoted the tobacco plant growth under greenhouse study and field experiments. Yip et al. (2003) showed that the tobacco line transformed with a neutral *Bacillus* phytase exhibited phenotypic changes in flowering, seed development, and response to phosphate deficiency. The transgenic line showed an increase in number of flowers and fruits, lesser seed IP6/IP5 ratio, and enhanced growth under phosphate-starvation conditions as compared to wild plants. These findings suggested that the over-expression of *Bacillus* phytase in the cytoplasm of tobacco cells shifts the equilibrium of the inositol phosphate biosynthesis pathway, thereby making more phosphate available for primary metabolism.

Transgenic Arabidopsis plant expressing an extracellular phytase from *Medicago truncatula*, led to significant improvement in organic phosphorus utilization and plant growth (Xiao et al. 2005). Overexpression of an acid phosphatase gene from Arabidopsis enhanced the P efficiency in soyabean roots (Wang et al. 2009). Using phytate as the sole source of phosphorus, dry weight and total phosphorus of the transgenic lines were higher than the control plants, suggesting the great potential of heterologous expression of phytase gene for improving plant phosphorus acquisition and for phytoremediation. The expression of plant derived phytase and phosphatase gene in white clover resulted in improved growth and P acquisition by the transgenic plants (Ma et al. 2009). Whether or not transgenic plants will be used for production of commercial phytases in the future, either directly for feeding or as a bioreactor, will depend on production costs and on public acceptance of green biotechnology. These aforementioned approaches can be applied as a strategy for boosting productivity in agriculture and horticulture.

Conclusions and future prospects

With increased concerns over phosphorus pollution in the areas of intensive livestock and availability of organic phosphorus in phytate form, phytases have immense potential in commercial and environmental applications. The tools of modern genetic engineering and molecular biology could be utilized for the development of foods and feeds with a higher iron, phosphorus and zinc content with improved bioavailability of the minerals and proteins. Furthermore, transgenic plants containing phytase gene from microbial source could also be used to improve soil

fertilization and nutrient uptake. The phytic acid content in food grains could be reduced using phytase to improve mineral absorption from food based diets. Transgenic plants with low phytic acid or expressing recombinant phytase could be a novel approach for lowering the rate of malnutrition with concomitant reduction in phosphorus content in animal waste.

However, further research is required to study the exact process of accumulation of phytic acid during seed development and effective implementation of this approach at the community level (Mendoza 2002). The scientists, working on different aspects of phytases should come together for the biotechnological development of an ideal phytase for improving animal nutrition, human health, and environmental protection.

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