Biotechnology, 4 (4): 333-340, 2005
ISSN 1682-296X

Role of Synthesis and Exudation of Organic Acids in Phosphorus Nutrition in Plants in Tropical Soils

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Abstract: Plants have evolved a diverse array of strategies to uptake adequate Phosphorus (P) under limiting conditions in tropical and sub-tropical soils, including modifications to root architecture (e.g. cluster roots in Proteaceae), carbon metabolism and membrane structure, exudation of low molecular weight organic acids, protons and enzymes and enhanced expression of numerous genes involved in low-P adaptation. These adaptations seem to be less pronounced in mycorrhizal-associated plants as mycorrhiza in roots significantly helps plants in P uptake at low P soils. The formation of cluster roots in concert with enhanced exudation of low molecular weight organic acids such as citric, oxalic, malic, fumaric, succinic etc. under P-stress by the non-mycorrhizal plants and the accompanying biochemical changes exemplify many of the plant adaptations that enhance P acquisition and use. Several biotechnological approach are now in progress to increase exudation of organic acids from the roots of economically important crop plants for sustainable crop production in tropical and sub-tropical soils.

Key words: Phosphorus nutrition, acid soils, phospho*enol*pyruvat carboxylase (PEPC) activity

INTRODUCTION

Phosphorus (P) is a limiting factor for crop yield in tropical and sub-tropical soils[1-4]. In these soils, P forms insoluble compounds with a number of di- and tri-valent cations (e.g., Al^{3+}, Fe^{3+}) and it is the least readily available nutrient in the rhizosphere[5,6]. Crop yield on 30-40% of arable land in the world is affected by P unavailability[7]. Concerns about depletion of high-grade phosphate rock, the necessary fertilizer raw material and cost of P fertilization in developing countries have stimulated the search for means of saving and utilizing P more efficiently[1,8-11]. Not only P fertilizer made from non-renewable resources but also up to 80% of the P fertilizer that is applied becomes unavailable to the plant due to its interactions in the soil[4].

Plants have evolved two broad strategies for P acquisition and use in nutrient-limiting environments: (1) those aimed at efficient use and (2) those directed towards enhanced acquisition or uptake[4,11]. Processes that use efficiently the acquired P involve decreased growth rate, increased growth per unit of P uptake, remobilization of internal inorganic phosphate (Pi), modification in internal carbon metabolism that bypass P-requiring steps and alternative respiratory pathways[11-13]. Alternative glycolytic reactions can bypass Pi or ATP-requiring steps of glycolysis under environmental stress conditions such as Pi starvation[14,15]. By comparison, processes that lead to increase P uptake include enhanced secretion of phosphatases and exudation of organic acids, changes in root morphology and enhanced expression of Pi transporters[5,11]. Current knowledge concerning clarification of the mechanism of plant tolerance to low P and/or excess Al is elaborately reviewed in this study.

Plants adaptation to phosphorus deficient soils: A wealth of data is available on the mechanism of P nutrition in different plant species under P deficiency. It is now clear that plant species and even genotypes within a species may differ widely in their P use efficiency[16,17]. The evolution of plants in environments where P availability is low in the rhizosphere has led to numerous adaptations required for the survival of plants[18]. Many researchers have identified the mechanisms of plant tolerance to low P concentration differs among species, which is mainly dominated by hereditary properties of plants[19]. Phosphorus use efficiency is related to the uptake efficiency of the plant, which is determined by both root-shoot ratio and absorption rate per unit of root (influx)[16]. The high P uptake efficiency under low P condition is regulated by various factors such as root

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morphology[19,20] and root activity[21], rhizosphere pH[22,23], root exudates[24,25] and mycorrhizal association[26]. Crops and forages that are genetically adapted to low P-supplying tropical soils are often characterized by a low P requirement and/or increased efficiency in absorbing P from soils of low P status[16,27]. It is established that increased uptake of Pi has been correlated with an increased number of high-affinity Pi transporters assembled in the plasma membrane[28-30].

Organic acid exudation from roots and phosphorus solubilization: Root induced chemical modification in the rhizosphere may be involved in the mobilization and exploitation of sparingly soluble phosphorus in soils. Root exudation of low molecular weight organic acids, mainly including acetic, aconitic, citric, fumeric, gluconic, lactic, malic, oxalic and succinic acids is enhanced in many plant species under P deficient condition[31-35]. It has been hypothesized that organic acids excreted by the roots may have been produced in the leaves and subsequently transported to the roots[36,37]. Hoffland[37] concluded that organic acid exudation was a highly effective strategy to increase phosphate uptake from rock phosphate.

Gardner *et al.*[24,39,39] found that the exudation of citrate by white lupin into the rhizosphere could increase P availability by mobilizing P from sparingly soluble Fe and Al phosphates. Ae *et al.*[25] found that pigeon pea, which is widely cultivated on Indian semi-arid tropical soils, had a strong ability to utilize Fe-bound phosphorus by exuding piscidic acid which chelated Fe and thereby released P from Fe phosphates. Thus, it is clear that different plant species or ecotypes may exude particular organic acids to mobilize different types of sparingly soluble P (Fe-P, Al-P or Ca-P)[40]. Ström *et al.*[41] found that acidifuge species could use oxalate to dissolve phosphate whereas calcifuge species could not. This may be the result of long terms adaptation to low-P soils in which Fe-P, Al-P or Ca-P are the dominant forms of soil P[42]. In an experiment in quartz sand culture supplied with either $Ca_3(PO_4)_2$ or $AlPO_4$, it was found that radish utilized P from $AlPO_4$ much better than from $Ca_3(PO_4)_2$, whereas the opposite phenomenon was found in rape[42]. The results demonstrated the role of a particular organic acid in mobilizing sparingly soluble P and were in accordance with the preferential growth of two plants on acid (radish) and calcareous (rape) soils in China. To confirm the hypothesis that plant species adapted to different soil environments would exude particular organic acids to mobilize specific forms of sparingly soluble P in soil, it is necessary to do further studies with more plant species (from acid and calcareous soils).

Variation in phosphorus use efficiency in native plants: There are two ways in which variation in P use efficiencies can arise: 1) the efficiency with which P is utilized to produce yield, i.e. the amount of P needed in the plant to produce one unit of dry matter[43]. This is often called internal P requirement and is the P concentration in plants to produce 80% of maximum yield. 2) the uptake efficiency of the plant, which is the ability of the root system to acquire P from soil and accumulate it in the shoots. This depends on the capability of roots to absorb P, the active life time of roots and on the amount of roots per unit of shoot. The components of uptake efficiency have been evaluated by Loneragan and Asher[44].

Plant adaptation to P-limited tropical soils can be partially attributed to inherent genotypic differences in P Use Efficiency (PUE)[16]. Phosphorus use efficiency is defined as the amount of total biomass and/or economic yield produced per unit of acquired P[45,46]. Such efficiency is directly controlled by plant traits and mechanisms related to basic metabolism[47], by pattern of partitioning and remobilization of P among different organs and tissues[48,49] and perhaps mostly by the capacity of the plant to accumulate dry matter owing to efficient utilization of P in plant metabolic processes. PUE is sometimes defined as the inverse of P concentration[50-52], which may be more useful in describing the current dynamics of P acquisition in relation to P utilization. PUE can also be defined as a response (measured in dry weight) for a given increase in P content during a certain period of time[53]. Practically all plants show an increase in PUE under conditions of P deficiency[54,55], because 1) a larger proportion of plant biomass is allocated to tissues with low P concentration (e.g. roots as contrasted with leaves or reproductive organs) and 2) P storage in vacuoles declines and structural and nonstructural carbohydrates increase[56,57]. A number of plant attributes have been identified which regulate the efficient utilization of acquired P uptake from low P-soils[49,58]. These plant attributes are, I) high dry matter yield per unit of P acquired, ii) growth duration and plant type, iii) partitioning of P between different pool within the plant iv) redistribution of previously assimilated P, v) leaf death rate and vi) partitioning of a greater proportion of biomass to harvestable yield[27]. Rao *et al.*[27] argued that improvement of PUE can be achieved at least by two major mechanisms: a) changing the partitioning of P among plant parts and b) increasing the metabolic efficiency of P at cellular level.

Genotypic variation in plant traits related to P acquisition and utilization has been observed in a number of crop and forage species[27,59,60]. Genetic studies indicated that high P response (i.e. higher dry weight

increase per unit of P applied) in white clover was dominant over low P response and narrow-sense heritabilities for P response were moderate[61]. The ratio of dominant to recessive genes in all white clove parents was approximately 2 for P response. Moreover, it was estimated that at least four individual or groups of genes are involved in the P response[61].

Function of phospho*enol*pyruvate carboxylase (PEPC) in organic acid exudation: Phospho*enol*pyruvate carboxylase (PEPC) is a cytosolic enzyme widely distributed in most plant tissues, green algae and microorganisms but not in a nimal cells[62]. Higher plant phospho*enol*pyruvate carboxylase (PEPC) is a multifaceted enzyme involved in various physiological contexts[63,64]. It is an important enzyme for the carbon economy of the cell, playing a central role in CO_2 fixation of C_4 and crassulacean acid metabolism (CAM) plants[65]. PEPC is a homotetrameric enzyme that catalyzes the β-carboxylation of phospho*enol*pyruvate by HCO_3^- in the presence of a divalent cation to yield Pi and oxaloacetate (OAA), which is readily converted to malate by NAD(P)-malate dehydrogenase[62]. PEPC activity produces OAA and malate that replenish the citric acid cycle, the so-called anaplerotic function, providing carbon skeletons for nitrogen assimilation[66]. In maize leaves, the addition of nitrate, ammonium and glutamine promotes the activation of PEPC expression[67,68], lending support to the proposal that PEPC activity plays an important role connecting carbon and nitrogen metabolism. Recently, several abiotic stresses (monovalent cations, drought, cold and hypoxia) induced expression of PEPC in roots of wheat seedlings[69]. These findings suggest that PEPC may play an important role in the adaptation of plants to environmental changes. Enhanced expression and activity of PEPC has been linked with P deficiency-induced biosynthesis and root exudation of carboxylic acids[70-73]. Under P-deficient conditions, the PEPC reaction, which liberates oxaloacetate and Pi, may have a function for Pi recycling in PEP catabolism as a bypass for the ADP- and Pi-depended pyruvate kinase[74].

When the maize C_4-PEPC gene was expressed in the rice lines, the photosynthetic rate was suppressed[75], indicating that this C_4-PEPC transgenic rice had no functional C_4 cycle. However, the photorespiratory rate was also suppressed in correlation with the activity of C_4-PEPC[76], pointing to a role of this enzyme other than the acquisition of carbon for photosynthesis. A reduction of the photorespiratory rate, presumably due to decreased sensitivity to O_2 caused by the introduction of C_4-PEPC, was also observed in tobacco[76]. C_3-PEPC is activated by hexose phosphates and triose phosphates, while it is inhibited by malate[77]. Phosphorylation alters its sensitivity to malate and it's Km for PEP[62]. Under high light condition, C_4-PEPC is activated by phosphorylation of a regulatory serine[62,78,79], which is controlled by a Ca^{2+}-independent PEPC kinase[79].

PEPC in C_3 plants has an additional role as compared to C_4-PEPC. In C_3 plants, PEPC competes with sucrose phosphate synthase (SPS) to direct the flow of photosynthetically fixed carbon towards the pool of TCA cycle intermediates[80], which it replenishes in the case of a shortage of phosphate[74,81] and/or an increased demand for amino acids. The roles of SPS and PEPC in carbon metabolism are different in soybean and rice. In soybean, the ratio of the activities of SPS and PEPC is lower than in rice[82]. As the carbon flow to organic acid and amino acid synthesis is greater in soybean than in rice, it was concluded that carbon allocation is regulated by the relative activities of SPS to PEPC. Thus C_4 type PEPC expression in C_3 plants offers opportunities to study the regulation of the synthesis of organic acids and amino acids.

PEPC expression and activity are associated with citrate accumulation in roots, enabling citrate excretion from roots to the rhizosphere[13,34,83-86]. PEPC has been suggested to direct the synthesis of organic acids from carbohydrates produced in the glycolytic pathway[72,73,87]. Attempts to increase the exudation of organic acids from roots by introducing enzymes such as citrate synthase, have yielded contradictory results. De la Fuente *et al.*[88] introduced a *Pseudomonas aeruginosa* Citrate Synthase (CS) genes into tobacco and papaya, causing both CS activity and citrate exudation to increase and Al tolerance was enhanced in the transgenic plants. However, another group could not repeat these findings using same tobacco lines, as well as other lines which expressed CS protein at much higher levels[89]. They also reported that Al tolerance in transgenic alfalfa lines expressing the *P. aeruginosa* CS protein was not enhanced compared with non-transgenic plant[89]. On the other hand, the overexpression of carrot mitochondrial CS in *Arabidopsis thaliana* resulted in increased CS activity and citrate exudation from roots and small enhancement was observed in Al tolerance[90]. The overexpression of PEPC in alfalfa did not result in a significant enhancement of organic acids accumulation and exudation from roots; on the other hand, overexpression of malate dehydrogenase dramatically increased acid accumulation and exudation[91]. Although the composition of organic acid exudation from roots differs between species, it is still important to determine whether PEPC activity can increase the synthesis and secretion of organic acids.

CONCLUSIONS

A diverse array of strategies including modification of root architecture, carbon metabolism and membrane structure, exudation of organic acids, protons and enzymes and enhanced expression of numerous genes involved in low-P adaptation. Several strategies have been used to manipulate the P efficiency in plants and /or to increase organic acid exudation from roots using the genetic engineering[92]. A wealth of data has been gathered on the mechanisms of P nutrition in different plants and environmental conditions. However, the precise understanding on genetic factors responsible for better adaptation of tropical crop plants to P deficient acidic soils (excess Al) is not clarified well. Physiological, biochemical and molecular studies of white lupin and other species response to P-deficiency have identified targets that may be useful for plant improvement. An appropriate biotechnological approach to increase exudation of specific organic acids from the roots of important crop plants would lead a break through for sustainable crop production in phosphorus limited tropical and sub-tropical soils.

ACKNOWLEDGMENTS

The first author is highly thankful to The Gen Foundation, UK for providing a scholarship during her Ph.D. study in Hokkaido University, Japan. A postdoctoral fellowship to M.T.I. by the Japan Society for the Promotion of Science (JSPS) is highly acknowledged.

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