Review

Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils

José López-Bucio, María Fernanda Nieto-Jacobo, Verenice Ramírez-Rodríguez, Luis Herrera-Estrella *

Departamento de Ingeniería Genética de plantas, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato, Apartado postal 629, 36500 Irapuato, Guanajuato, Mexico

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Abstract

During the last 20 years increasing experimental evidence has associated organic acid metabolism with plant tolerance to environmental stress. Current knowledge shows that organic acids not only act as intermediates in carbon metabolism but also as key components in mechanisms that some plants use to cope with nutrient deficiencies, metal tolerance and plant–microbe interactions operating at the root–soil interphase. In this review we summarize recent knowledge on the physiology and occurrence of organic acids in plants and their special relevance concerning nitrate reduction, phosphorus and iron acquisition, aluminum tolerance and soil ecology. We also discuss novel findings in relation to the biotechnological manipulation of organic acids in transgenic models ranging from cell cultures to whole plants. This novel perspective of organic acid metabolism and its potential manipulation may represent a way to understand fundamental aspects of plant physiology and lead to new strategies to obtain crop varieties better adapted to environmental and mineral stress. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. The ubiquity of organic acid metabolism

Organic acid metabolism is of fundamental importance at the cellular level for several biochemical pathways, including energy production, formation of precursors for amino-acid biosynthesis and at the whole plant level in modulating adaptation to the environment.

A striking feature of plant tissues is that the total content of organic acids is higher than in other organisms. The composition of organic acids that accumulate varies depending upon the species, age of the plant and the tissue type. The high accumulation of organic acids in plant tissues is most probably due to their important role as photosynthetic intermediates. However, organic acids have a potential role as metabolically active solutes for the osmotic adjustment and the balance of cation excess. Organic acids also participate as key components in the mechanisms that some plants use to cope with nutrient deficiencies, metal tolerance and plant–microbe interactions operating at the root–soil interface.

Organic acids are mainly produced in mitochondria through the tricarboxylic acid or Krebs cycle and to a lesser extent in the glyoxysome as part of the glyoxylate cycle. Because of the catalytic nature of the Krebs cycle, organic acids are present only in very small pools in the mitochondria and are preferentially stored in the vacuole. It is unlikely that the concentrations of the Krebs cycle intermediates in plants are appreciably different...
from those in the mitochondria of other living organisms. As already mentioned, the accumulation of certain organic acids in plant tissues or their excretion by roots must conform to their participation in specific physiological functions [1](Fig. 1).

2. Organic acids as photosynthetic intermediates

The assimilation of CO₂ from the atmosphere by plants involves three basic mechanisms: C₃ photosynthesis, C₄ photosynthesis and Crassulacean Acid Metabolism (CAM). The occurrence of organic acids as photosynthetic intermediates is a widely described phenomenon that characterizes C₄ and CAM species. In CAM plants, including the Crassulaceae and more than 20 other families, CO₂ is acquired at night and incorporated into the carboxyl group of an organic acid (normally malic acid), which accumulates in the vacuole of the cell in which it is synthesized. During the following photoperiod, the accumulated organic acid is de-carboxylated to produce CO₂ which is consumed by the reactions of the photosynthetic carbon reduction cycle. The participation of malic acid as an intermediate of CO₂ fixation has been extensively studied in relation to stomatal aperture and water use efficiency. There is no doubt about the important ecological significance of organic acid metabolism in CAM plants, allowing them to survive in arid regions, such as deserts and rocky outcrops [2,3].

Besides malic acid, citric acid production may have a role as a CO₂ reservoir in CAM plants. In several CAM plants, such as Bryophyllum calycinum, Clusia minor, Clusia rosea, Notonia petrea, and Cissus quadrangularis, high concentrations of citric acid, sometimes exceeding 200 mM, have been reported [4]. In Clusia minor the intracellular transport of citrate has been reported. In this species, during the dark period after malate formation by phosphoenolpyruvate carboxylase, citrate is synthesized from malate or oxaloacetate in the mitochondria and is then exported to the cytoplasm [5]. Besides acting as a CO₂ reservoir, the possible ecophysiological relevance of citric acid accumulation in CAM plants has not yet been determined.

The universal importance of organic acids in plant physiology is often associated with their versatility in C₃ and C₄ plants in which a plethora of adaptive responses mediated by these molecules are now being investigated.

3. Organic acid accumulation in monocots and dicots

Levels of organic acids vary between species, cultivars, or even between individual tissues of a plant when grown under identical conditions. Moreover, the biosynthesis, accumulation, transport and root exudation of organic acids is dramatically increased in response to environmental stress.

In several studies, the apoplastic accumulation of organic acids has been found to be associated with cation transport in roots and vascular tissues of herbaceous plants and trees. In the apoplastic solution from the leaves of several Fagaceae (Quercus ilex, Quercus virginiana and Fagus sylvatica), the total concentration of organic acids ranged between 1.5 and 2 mM of which quinate, malate and oxalate were the most abundant. The fact that high concentrations of inorganic cations (4 to 17 times higher in quantity than anions) were also present in these apoplastic fluids led to the proposal that organic anions play a role in buffer-
ing the excess of nutrient cations [6]. High levels of malate have been reported in the apoplastic sap in Saccharum spp. and Hordeum vulgare and in lower amounts in the dicotyledonous Spinacia oleracea and Gossypium hirsutum [7–9].

Organic acids such as succinate, citrate, malate, oxalate, lactate and fumarate are known to be transported from the roots with the transpiration stream. A diurnal variation in the concentration of cations and succinate, citrate and fumarate was observed in the xylem sap of Ricinus communis. In Q. ilex and Lagerstroemia indica, the concentration of these organic ligands in the xylem reached a maximum at night. The accumulation of organic acids in the xylem when transpiration stops may suggest a diurnal exchange between the leaf apoplast and symplast [10–12].

Organic acid transport in the transpiration stream has also been correlated with the transport of micronutrients. Formation of metal–citrate complexes resulted in more zinc passing through the excised stem of Pinus radiata, copper in Pyrus stems and iron in dicotyledonous. For instance, it has been found that these complexes are more efficiently transported in the xylem by a lower adsorption to the negatively charged vessel walls and a diminution of lateral escape [13–15].

Another probable significance of organic acid accumulation is their participation in the balance of charges formed during the extensive metabolism of anions such as nitrate (NO₃). Nitrate is the predominant form in which inorganic nitrogen is taken up by plant roots. Once inside the cell, NO₃⁻ is converted to the reduced cation NH₄⁺, which is utilized in aminoacid biosynthesis.

During rapid vegetative growth, the rates of nitrate reduction, carboxylate synthesis and amino acid synthesis are high. Nitrate reduction implies the formation of toxic alkaline ions (1 mol of hydroxide for every mol of nitrate reduced). These ions can not be efficiently expelled from the cell, therefore, to maintain pH homeostasis, the plant synthesizes organic acids (principally citrate and malate) in leaves which are transported to the sites where NO₃⁻ reduction is occurring [16,17].

Organic acids have also been found to stimulate nitrate uptake. Nitrate uptake rates by roots of intact soybean plants is stimulated by malate, which moves down the phloem and accumulates in the root [18].

The relationship between nitrate assimilation and organic acid metabolism has been elegantly explored in mutant plants with low nitrate reductase activity (Nia 30 mutants), which accumulate large amounts of nitrate. When wild type tobacco plants are grown on low nitrate, a large decrease in malate and citrate levels has been documented. These organic acids increased three–ten-fold in plants growing on high nitrate (12mM). In nitrate reductase affected plants, the transcripts for PEP carboxylase, cytosolic pyruvate kinase, citrate synthase and NADP-isocitrate dehydrogenase are especially increased. This increase in mRNA levels was accompanied with the accumulation of malate and citrate in leaves and roots. From this work, it has been suggested that nitrate acts as the signal to initiate coordinated changes in carbon and nitrogen metabolism, and organic acid production [19].

Understanding the relationship between nitrate uptake and utilization and organic acid metabolism, could be an important avenue to enhance nitrogen fertilizer use by genetic engineering.

4. Organic acids in plant adaptation to soil stress

Most agricultural soils are affected by edaphic factors that drastically limit plant growth and productivity. Soil chemistry is strongly related to pH and an increase or diminution in this critical factor often forces plants to cope with heavy metal toxicity, bicarbonate excess, deficiencies or toxicities of calcium and micronutrients, and low nitrogen and phosphorus availability [20].

Plants growing in alkaline soils where calcium is abundant, often reduce the excess of cellular calcium by combining it with organic acids. This is crucial for survival under these soil conditions because high concentrations of Ca interfere with essential cell processes, such as calcium-dependent signaling, phosphate-based metabolism and cytoskeletal dynamics [21]. In fact, the precipitation of calcium in the form of calcium oxalate crystals has been reported for several plant species. These crystals accumulate selectively in vacuoles of roots, stems, leaves, flowers, fruits and seeds [22,23].

Soil fertility and ion toxicity are the major constraints for the use of marginal soils that represent the most important alternative for the agricul-
tural expansion necessary to meet the future demand for food. In acid soils, which account for about 40% of the world’s arable land, high phosphorus fixation and aluminum poisoning drastically limit the expansion of agriculture. In alkaline calcareous soils of arid and semiarid regions, which cover more than 25% of the earth’s surface, low P and Fe availability severely limit plant productivity [24].

Plant nutrition problems, often associated with soil chemistry, necessitate the use of high quantities of fertilizers to maintain a sustainable production. However, the indiscriminate utilization of these compounds has been associated with severe ecological disturbance [25]. Because of the strong selective pressure typical of marginal lands, some plant species are nevertheless well adapted to grow and reproduce under these unfavorable conditions. The research conducted over the past 20 years has shown that one essential mechanism by which plants tolerate metal and nutritional stress occurs at the soil–root interface and involves organic acid exudation [26].

It has been well documented that plant roots exude a variety of organic compounds, whose role in plant–soil interactions is beginning to be elucidated. Root exudates contain components that play important roles in nutrient solubilization (e.g. organic acids, phytosiderophores and phenolics), restricting the passage of toxic metals across the root (e.g. citrate, malate, small peptides) and attracting beneficial microorganisms (e.g. phenolics, organic acids and sugars). Often, the excretion of these organic molecules increases in response to soil stress [27].

In the following paragraphs, we illustrate the importance of root excretion in plant adaptation to soil stress by discussing the well-characterized effects of organic acid exudation in mineral nutrition, aluminum tolerance and plant-microbe interactions.

5. Phosphate uptake

Phosphorus (P) is an essential nutrient for plant growth, development, and reproduction that forms part of key molecules such as nucleic acids, phospholipids, ATP and other biologically active compounds. After nitrogen, P is considered the most important nutrient limiting agricultural production. Phosphate, the anionic form in which P is assimilated by living organisms is extremely reactive and is only available for plant uptake at a narrow range of neutral soil pHs. In acid soils, P forms low solubility molecules with aluminum (Al) and iron (Fe), whereas in alkaline soils, it combines efficiently with calcium (Ca) and magnesium (Mg) to form sparingly soluble phosphate compounds. For this reason, although the total amount of phosphorus in the soil may be high, in most cases it remains unavailable for plant uptake [28,29].

It has been well documented that, because of its high affinity for di-and tri-valent cations, citrate and other organic acids can displace P from insoluble complexes, making it more soluble and thus available for plant uptake [30,31]. Evidence that organic acid excretion could play an important role in phosphate-solubilization was first obtained when researchers found that the roots of certain plant species grown under P deficient conditions contain higher concentrations of organic acids than non-stressed plants. For instance, dicotyledonous plant species belonging to the Brassicaceae and Proteaceae families, and lupin (Lupinus albus), which are well known for their ability in mobilizing P from the soil, significantly increase the content of organic acids in their exudates in response to P stress [32].

The roots of rape (Brassica napus) excrete citric and malic acids into the rhizosphere and solubilize P from rock phosphate. In Proteaceae and Lupinus albus, when growing under P starvation, the formation of citrate overproducing cluster roots (proteoid roots) has been described. These specialized roots are directly responsible for the 13–40-fold increase in organic acid excretion in response to P stress. The ability to exude large amounts of organic acids has been associated with the efficient use of sparingly soluble calcium phosphate compounds especially abundant in alkaline soils. Similarly, citric acid secreted by chickpea (Cicer arietinum) roots enhances the solubility of P from vertisols where a high proportion of phosphate is associated with cations [33–37].

In an interesting ecological work, the relation between organic acid exudation and the adaptive advantages of calcicole plants (plants adapted to alkaline soils) in terms of plant nutrition was examined. Interestingly, the quantity of organic acids released by the roots of calcicoles was sev-
eral-fold higher than those of plants not well adapted to grow in alkaline soils. This research provided strong evidence that the ability of calci-coles to grow in alkaline soils is due to their capacity to extract phosphate and iron through organic acid exudation [38,39].

The levels of enzymes involved in organic acid biosynthesis often increase in response to P starvation; this is the case with PEP carboxylase and citrate synthase in lupin and rape plants, and PEP carboxylase in pea, tomato and Brassica nigra. This increase in enzyme levels has been related to a higher amount of organic acids being siphoned off from the TCA cycle for root exudation, which in turn leads to more efficient P acquisition capacity [40–44].

6. Iron nutrition

Iron (Fe) is a micronutrient that limits plant growth in calcareous soils, in which it forms part of oxyhydroxide polymers with extremely low solubility. The main symptom of Fe deficiency is the appearance of interveinal chlorosis in young leaves. This symptom is more frequent and pronounced in crop plants growing in alkaline soils, from which the term “lime-induced chlorosis” is derived [45,46]. For several crop plants, including maize and sorghum, chlorosis due to iron deficiency remains an economically important problem in spite of decades of research [47].

The participation of organic acid excretion in the mechanisms that allow cells to cope with a low iron supply has been reported in diverse organisms ranging from bacteria to plants. For instance in Eschericia coli and Pseudomonas putida, under iron-limiting conditions ferric citrate activates the transcription of genes encoding a ferric-citrate transport system [48].

Many dicotyledonous plants respond to Fe-deficiency stress by: (i) release of hydrogen ions by their roots, (ii) reduction of the soil-abundant Fe$^{3+}$ to the more accessible Fe$^{2+}$ iron cation; and (iii) exudation of organic acids. Recently, it has been proposed that root exudation of citrate may play an important role in supplying Fe to dicotyledonous plants. Studies in which different varieties of soybeans, tomatoes and corn were tested for Fe uptake efficiency showed that citric acid accumulation is always more pronounced in the Fe-efficient genotypes [49–51].

7. Aluminum tolerance

Aluminum (Al) toxicity is a global problem that limits crop productivity on acid soils. Al is the most abundant metal in the earth’s crust where it remains mostly insoluble. However, in soils with
pH lower than 5.5 (acidic soils) the phytotoxic species Al$^{3+}$ is solubilized to levels that drastically inhibit root growth and as a consequence, crop yield [55]. Although temporal alternatives to correct soil acidity exist (i.e. liming), Al toxicity remains one of the most important agricultural problems worldwide. In spite of many years of research efforts aimed at resolving this problem, little is known about the fundamental mechanisms responsible for Al toxicity and resistance [56].

The most easily recognized symptom of Al toxicity is the inhibition of root growth. In addition to the root growth inhibition caused by Al$^{3+}$ exposure, at the plant and cellular level a myriad of different symptoms has been described both in shoots and roots. Some of these changes resemble plant nutrient deficiencies, such as those of P stress [57–60].

It has been well documented that several native and cultivated species exhibit significant genetic variability in their response to Al toxicity. Two basic strategies by which plants can tolerate Al have been proposed: first, the ability to maintain Al outside the root and second, mechanisms that allow plants to internally tolerate toxic concentrations of Al.

Several hypotheses have been tested to explain how plants could avoid Al from entering into the root. From the insights obtained, it has been proposed that a tolerant phenotype may include mechanisms involving rhizosphere alkalinization, low cell-wall cation-exchange capacity, Al exclu-
sion by secretion of chelating compounds and active Al\(^{3+}\) efflux across the plasma membrane [61–64]. Increasing experimental evidence supports that among these, the most ubiquitous tolerant mechanism in the plant kingdom is the exclusion of Al from the root apex via root exudation of organic acids. When these ligands are released into the rhizosphere, they can effectively chelate Al\(^{3+}\) and prevent its entry into the root. In several plant species, increased Al resistance correlates with higher rates of citrate and malate exudation. In snapbean, maize and Cassia tora the Al tolerant phenotype is associated with secretion of citrate, whereas in wheat the resistant response depends on Al-inducible release of malate [65–68].

The effectiveness of citric acid in alleviating Al toxicity has also been demonstrated in wheat, in which the addition of citrate to solutions containing toxic levels of Al, reverses the inhibition of root growth. Citric acid forms a strong chelate with Al, typified by a stability constant of \(5 \times 10^{12}\) which is about 700-fold greater than the corresponding value for the Al–malate complex [69].

It is possible that different processes affecting organic acid excretion modulate the plant response to soil stress. Al-induced exudation of organic acids may involve the induction of organic acid biosynthesis and/or the activation of high-affinity organic acid transporters to enhance their adaptive capacities [70]. Recently, Ryan et al. (1997) described an anion channel in the plasmalemma of protoplasts isolated from roots of wheat. This channel is specifically activated by aluminum and was detected in protoplasts isolated from the root apex but not in protoplasts from mature root tissue [71]. This specificity for organic acid exudation in response to Al stress has also been documented in Cassia tora in which exposure to Al\(^{3+}\) induces secretion of citric acid after 6h of treatment. In this species, exposure to 50 \(\mu\)m of either lanthanum (La\(^{3+}\)) or Ytterbium (Yb\(^{3+}\)) did not induce the secretion of citric acid. Data from the same research group has shown that the aluminum tolerant juxai cultivar of buckwheat (Fagopyrum esculentum M.) secretes oxalic acid from its roots specifically and quickly in response to Al stress [72].

The exudation of organic acids in aluminum tolerant plants could represent an evolutive convergence directed to cope with Al, because organic acids effectively protect the root from Al toxicity. Alternatively, it may be part of a more general adaptive mechanism to increase the capability of native species to survive in acid soils that also accounts for other important traits such as P use. The validity of this hypothesis remains to be investigated.

8. Organic acids in plant-soil ecology

Most previous research has focused on the role of organic acids as photosynthetic intermediates, or in nutrient uptake and Al-tolerance; however, a role for root exudates in general plant–soil ecology is now being investigated.

It has been documented that by releasing organic compounds, plant roots create a zone of intense nutrient exchange and microbial activity. This zone of soil, in which microorganisms are greatly influenced by root exudates, leads to the formation of microbial gradients where the maximum number and diversity of individuals occur in the vicinity of the root and decline away from it. This unique soil environment is termed the rhizosphere [73].

The presence of high numbers of bacteria and fungi in the rhizosphere can influence several aspects of plant growth and crop productivity. Some of these microorganisms may solubilize nutrients such as P and Fe, others may affect root morphology and shoot–root ratio by producing plant growth regulators [74].

Studies on the nutritional requirements of rhizosphere microbial populations suggest that the number and activity of soil microorganisms rely greatly on simple organic compounds. These studies have linked organic acid exudation with the attraction of plant-beneficial soil bacteria. Krotsky et al. (1986) showed that a sorghum cultivar efficiently colonized by N\(_2\) fixing free-living bacteria released more malic, fumaric and succinic acid than a less active cultivar [75,76]. Rennie (1980) reported that the addition of sugars and organic acids to maize inoculated with N\(_2\) fixing Azospirillum brasilense promoted the incorporation of atmosphere-derived nitrogen in the plant. Succinate and malate stimulated nitrogen fixation more than sucrose [77]. More recently, it was found that aluminum tolerant wheat cultivars that produce high concentrations of low molecular dicarboxylic
acids had higher associative nitrogen fixation rates than non-tolerant cultivars [78].

The adequacy of organic acids as carbon and energy sources has been demonstrated with studies on other microbial species. Some *Campylobacter* sp isolated from roots of *Spartania alterniflora* were found to efficiently metabolize amino and organic acids [79]. In legume-Rhizobium symbiosis, the preferred substrate taken up by the bacteroids from the host is malate, which may be oxidized to oxaloacetate by malate dehydrogenase or may be converted to acetyl CoA by the malic enzyme and pyruvate dehydrogenase. Further oxidation of acetyl CoA in the tricarboxylic acid cycle can generate the large amount of energy required by the nitrogenase reaction [80].

In this line of research, it has also been demonstrated that citric acid can influence nitrogenase activity. Nitrogenase is the enzyme that catalyzes the ATP-dependent reduction of N₂ to NH₃. The rate of acetylene reduction by *Azospirillum brasilense* is considerably increased when associated with wheat roots. Such an increase in nitrogen fixing activity has been correlated to a specific root exudation behavior. In *Klebsiella pneumoniae* homocitrate and citrate containing nitrogenases have been identified. These organic acids may be acting in the uptake and stability of metal ions that form the prosthetic group of the enzyme [81,82].

Whether a direct link exists between aluminum tolerance and the ability of certain plants to associate with *Azospirillum* via organic acid exudation behavior is difficult to conclude. Further work is certainly needed to confirm the functional significance of these interesting findings.

### 9. Genetic manipulation of organic acid biosynthesis

Most of the studies aimed at determining the role of organic acid excretion have been carried out by comparing different plant species or non-isogenic lines of the same plant species. Plant transformation allows the production of genetically identical plants that differ only in one or a few genes. Taking advantage of this technology, our research group produced transgenic plants with an enhanced capacity to synthesize and excrete citrate. We reasoned that by overproducing citrate (one of the most powerful cation chelators in the organic acid group) the actual relevance of organic acids in several aspects of the plant–soil relationship could be elucidated.

To produce citrate-overproducing plants (CSb) plants, the coding sequence of the bacterial citrate synthase gene was placed under control of the 35S CaMV promoter and the nopaline synthase 3’-end sequence (35S–CSb). This construct was used to transform tobacco and papaya plants. To determine whether the expression of a citrate synthase in plant cells leads to an increase in their citrate content, total and root extracts of transgenic lines were examined by HPLC and compared to control plants. It was found that the tobacco lines expressing the 35S–CSb construct had up to ten-fold higher levels of citrate in their root tissue. The amount of citrate exuded by the roots of these transgenic lines was also increased up to four-fold as compared to control plants [83].

The first step we made in characterizing the novel transgenic CSb lines was to determine whether these plants were tolerant to aluminum. Several experiments were carried out to monitor the growth of transgenic and control plants in nutrient media containing toxic concentrations of Al. It was found that citrate-overproducing plants could tolerate a ten-fold higher Al concentration than control plants. In order to examine whether citrate overproduction also protects the root from Al damage, CSb and control seeds were germinated on media containing toxic levels of this metal. At concentrations higher than 300 μM, control seeds germinated, but did not develop a root system, and at low Al concentration (50–75 μM) root growth was only slightly affected, but root hair development was severely impaired. In all cases, CS transgenic lines were more resistant to these effects [83] (Fig. 2B).

Hematoxylin staining was used to test the penetration of Al inside the roots of CSb plants. Hematoxylin, originally colorless, turns violet in the presence of Al. This method has been extensively used to assess aluminum tolerance in plants. When tested by this method, the roots of CS lines showed less staining than did controls when exposed to high concentrations of Al, indicating that lower amounts of Al penetrated the tissue, including the root tip and root hairs. Taken together these results demonstrate that citrate overproduction can effectively protect the plant by restricting the toxic Al to the exterior of the root. This work
shows that organic acid overproduction could be a promising alternative to obtain transgenic varieties more adapted to grow in acidic soils.

Besides their increased tolerance to Al, two important conclusions can be highlighted in relation to the overproduction of citrate: (i) oxaloacetate and acetyl-CoA, the substrates of citrate synthase, are available in the plant cytoplasm; and (ii) an increased synthesis of citrate leads in turn to a higher efflux of citrate. This implies that active organic acid channels are normally functioning in plant roots [83].

Since the root exudation of organic acids has been strongly related to phosphate acquisition in several plant species, the efficiency for P acquisition of the citrate-overproducing tobacco lines was evaluated.

To compare the productivity of citrate overproducing and control lines, plants were grown in a natural alkaline soil (pH 8.4) with low P availability. In this soil, the growth of control plants was severely restricted and after 6 months they failed to flower and to produce seeds, whereas transgenic plants were able to grow and reproduce (Fig. 3A). The inability of control plants to complete their life cycle in this calcareous soil is probably a reflection of calcifuge behavior in which the inability to mobilize P is of central importance [84].

To confirm the relationship between P deficiency and plant growth, the growth of the CS plants in the same alkaline soil with different sources of P was evaluated. In treatments with high levels of soluble P (108 ppm of NaH₂PO₄), no differences in size, shoot and capsule dry weight were detected between transgenic and control plants (Fig. 3C). However, at sub-optimal levels of soluble P (10 and 22 ppm), the citrate overproducing plants had more shoot and capsule biomass and accumulated more P in their tissues than did controls (Fig. 3B). In addition, transgenic plants produced significantly higher amounts of capsule biomass when supplied with a sparingly soluble Ca–P source (hydroxyapatite) [84].

The finding that CSb plants grow efficiently in alkaline soils with low soluble P content, the better use of Ca-P compounds and the higher accumulation of P in their shoots demonstrate the importance of citrate exudation as an adaptive trait of plants to survive under P limiting soil conditions [84]. Other important agricultural considerations potentially related to citrate overproduction in plants such as the uptake and transport of iron, and the effect of citrate exudation on soil microbial populations are now being investigated by our group using CSb lines as a model system.

In related research, the relevance of organic acid excretion in relation to Al tolerance and P acquisition in plants has been addressed using cells in culture. In acidic conditions, a citrate overproducing carrot cell line selected to be tolerant to aluminum was evaluated for use of sparingly soluble P sources. When insoluble Al-P (2.0 mM) was supplied as a sole source of P, the selected cell line, but not the normal cells, grew as normally as cells supplied with soluble Na-P. This work strongly suggests that citrate excretion confers a dual advantage to plants in acid soil, conferring tolerance to Al as well as enhancing insoluble Al-P use.

Fig. 3. Growth of control (1522) and citrate overproducing (CSb-4,CSb-18) plants under suboptimal and optimal phosphate nutrition. A). 8 ppm, B). 22 ppm, C). 150 ppm of NaH₂PO₄. CS transgenic tobacco outperformed the control when growing at low applications of P in alkaline soils.
Subsequent work of this group showed that the citrate overproducer line had indeed an altered citrate synthesis metabolism characterized for higher levels of mitochondrial citrate synthase than wild type cells [85,86].

The possibility to induce organic acid production by overexpressing mitochondrial citrate synthase has also been tested. A mitochondrial citrate synthase of Arabidopsis thaliana was introduced into carrot (Daucus carota) cells by Agrobacterium tumefaciens. Several transgenic carrot cell lines that produced the Arabidopsis CS polypeptides and had high CS activity were identified. The increase in CS expression resulted in an enhanced capacity of phosphate uptake from insoluble sources of P in these transgenic cells [87]. More recently this research group has shown that transgenic Arabidopsis plants that express high levels of the carrot citrate synthase have an enhanced aluminum-tolerance and are more efficient in the utilization of insoluble sources of P (Dr. H. Koyama personal communication).

It has been reported that organic acid excretion by lupin plants constitutes a drain of 5–25% of the total fixed C, however this does not appear to significantly affect dry matter production. This fact has also been confirmed in transgenic tobacco plants that overproduce citrate, which grow efficiently even at high levels of P-fertilization [84].

The insights obtained from transgenic models highlight the potential of organic acid manipulation to generate novel crops more efficient in the use of soil-P and well adapted for growth in marginal soils [88].

10. Perspectives

Recent estimations show that an additional 446 million hectares of soil must be cultivated in order to satisfy the alimentary demands of the eight billion people that will inhabit the planet in the 3rd decade of the next millennium. It is clear that the greatest potential for agricultural expansion is marginal soils in which crop production has been limited and where land is still available for cultivation. Classical examples of the attempts to open marginal soils to agriculture has been the use of acid soils with adequate supplies of water, but in which the high content of aluminum drastically reduces plant production. Another case is the alkaline soils of semi-arid climates, which have traditionally sustained traditional rain-fed cultivation, however low P or Fe availability (even when fertilizers have been used) have strongly limited a more productive crop management [89,90].

The knowledge of organic acid biosynthesis, their universal occurrence in plants and the effectiveness of organic acid exudation in conferring Al tolerance and enhanced P uptake from sparingly soluble P compounds makes the organic acid pathway a promising target to develop transgenic varieties better adapted to grow in marginal soils. A major opportunity is to modify the quality and quantity of these organic compounds to target the rhizosphere, in this way genetic manipulation can contribute to a better understanding of the specific carbon substrates preferred by beneficial microorganisms, such as N2-fixing soil bacteria and mycorrhizal fungi (Fig. 4).
Currently, many different genes involved in organic acid biosynthesis have been cloned from several organisms. Through genetic engineering, it could be possible to overexpress these genes under the regulation of strong and tissue specific plant promoters. The incorporation of transgenic crops into integrated plant management and land use strategies, could represent a promissory option for agricultural expansion with a lower environmental cost. It is certainly a research priority to achieve a more sustainable agriculture for future generations.

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