Plant and microbe genomics and beyond: potential for developing a novel molecular plant nutrition approach

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Received: 9 August 2014 / Revised: 21 June 2015 / Accepted: 26 August 2015 / Published online: 18 September 2015
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Abstract Classical soil science approaches have enabled us to establish basic principles of how the soil system functions and have answered numerous practical agricultural application questions. In recent years, efforts have been refocused on better understanding, managing and benefiting from this system that contains one of the most complex biological communities of the planet. Soil biology is seen as being at the center of scientific research of this century, with novel research objectives and goals being set. In addition, plant nutrition has enabled us to understand nutrient uptake, transport and mobilization mechanisms in plants, and both disciplines have converged on the area of microorganism-mediated plant nutrition. The challenge for these scientific areas is to identify microorganism communities and the roles they play in their habitats, as well as the mechanisms that plants have to make better use of nutrients. Genomics and metagenomics, along with microbiological techniques, are contributing greatly to advances in our understanding of living systems that exist in the soil and their interaction with plants. For its part, molecular plant nutrition has made significant progress in understanding the use of nutrients by plant cells, and has identified molecular mechanisms that can improve nutrient use efficiency. Together, molecular soil microbiology and

molecular plant nutrition are projected to be a driving force in agriculture and sustainable food production in the coming years. Herewith, we aim to integrate recent literature on basic and applied research concerning plant and microbe genomics in terms of their potential for developing a novel molecular plant nutrition approach, with special emphasis on nitrogen, potassium and phosphorous as the major macronutrients for crop plants.

Keywords Life sciences · Molecular microbiology · Nutrients · Soil science · Innovation

Introduction

In the traditional approach, soil science includes studies in physics, chemistry, mineralogy, biology, and taxonomy to understand the processes that affect nutrient retention and release, the impact of pollutants and water availability, all of which significantly influence the soil–plant system. This interdisciplinary area also encompasses the study of the diversity of organisms in the soil and in the rhizosphere of plants, as well as their contribution to the recycling of organic material and their functions in regulating the availability, mobility, and use of nutrients by plants (Kutílek and Nielsen 2015). Importantly, the rhizosphere soil microbiome is influenced by a complex and nested array of factors at varying spatial scales, including plant community, plant host, soil edaphic and microbial taxon and community characteristics (Bakker et al. 2014). By studying such processes, it is possible to provide crucial information related to soil use and management that results in improved soil fertility and quality based on physical, chemical, and biological aspects; at the same time, such studies lead to the generation of practices aimed at better soil utilization, protection,

Communicated by A. K. Kononowicz.

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conservation, rehabilitation and remediation (Lindbo et al. 2012). Therefore, efficient integrated plant nutrition management represents one of the most critical factors for achieving sustainable agriculture and food security.

Plant nutrition is related to mineral metabolism, the physiology of crop yield, the optimal use of organic and mineral fertilization, and plant quality and health. According to FAO (2006), plant nutrient requirements can best be supplied through an integrated use of diverse nutrient resources, taking advantage of a combined and harmonious application of organic, mineral and biofertilizer nutrient inputs. This approach may serve the concurrent needs of food production, and economic, environmental and social viability. Consequently, this concept strives to ensure ecosystem sustainability against nutrient mining and degradation of soil and water resources, and enable a reasoned way to balance efficient use of limited or finite fertilizer resources. Among major plant nutrients, nitrogen (N) is abundant in the air, while potassium (K) deposits are considerable (López-Arredondo et al. 2014; Véry et al. 2014); however, phosphorous (P) reserves will become scarce (Cordell et al. 2011). Although a general claim has explicitly drawn attention to the challenges presented by global phosphorus scarcity (Cordell and White 2011), there is still a lack of policy debate and action concerning strict rules for recycling and efficient use of P. To bridge these gaps, a general scientific and political effort to provide support to the ongoing attempts directed at enhanced and sustainable agricultural production based on integrated plant nutrient management is needed.

Plant nutrition has made significant progress, thanks to advances in molecular biology (von Wirén 2011). Improved understanding of plant–microbe interactions and advances in molecular biology applied to soil microbiology have led to the emergence of areas such as molecular microbiology, soil metagenomics and molecular plant nutrition (Rincon-Florez et al. 2013).

In this review, we analyze the most salient advances in molecular technologies used for the study of soil biology and plant nutrition, and discuss areas of opportunity that should be covered with the support of the genomic sciences to make more efficient use of nutrient sources, under a sustainable agriculture approach. Unfortunately, it was not possible in this review, due to space limitations, to reference all of the valuable contributions made in this area.

Molecular techniques for the study of functional groups of soil microorganisms

Soil microbial activity is critical to the maintenance of life on Earth, as it is responsible for the mineralization of organic material, influencing nutrient recycling and

availability and consequently plant bioremediation and nutrition processes. Microorganisms that inhabit the soil make up very diverse populations and with recent advances in molecular biology even more complex levels of diversity have been discovered (Rastogi and Sani 2011).

Currently, through techniques that omit the use of artificial media for microbiological studies, it is possible to analyze the microbial composition of certain environments, especially when it comes to microorganisms that cannot be cultured *ex situ*. A recent review published by Rincon-Florez et al. (2013) describes three groups of culture-independent techniques for analyzing the biomass, diversity and catabolic activity of microbial communities. The first group encompasses eight techniques based on PCR: DDGE/TGGE (Kirk et al. 2004; Dowd et al. 2008; Cleary et al. 2012), T-RFLP (Kakavas et al. 2008), SSCP (Nocker et al. 2007), ARISA/RISA (Kirk et al. 2004), LH-PCR (Okubo and Sugiyama 2009; Mills et al. 2007), RAPD (Fritsch and Rieseberg 1996; Newbury and Ford-Lloyd 1993), ARDRA (Nocker et al. 2007) and q-PCR (Smith and Osborn 2009). The second group includes six techniques not based on PCR: CFE (Alessi et al. 2011; Ocio and Brookes 1990), PLFA (Zhang et al. 2007), FDA (Adam and Duncan 2001; Green et al. 2006), SIP (Dumont and Murrell 2005), DNA arrays (Li and Liu 2003) and FISH (Moter and Göbel 2000). Finally, high-throughput sequencing technologies comprise six techniques: 454 (Mardis 2008), Illumina (Loman et al. 2012), SOLiD (Magi et al. 2010; Shokralla et al. 2012), PGM (Egan et al. 2012; Loman et al. 2012), HeliScope (Magi et al. 2010; Shokralla et al. 2012) and SMRT (Schadt et al. 2010). Some of these techniques are also applied to the study of plant genomics.

Since most soil prokaryotes are not able to grow on artificial culture media (Stewart 2012), beginning with the discovery of molecular markers such as phospholipid fatty acids (PLFA) and nucleic acids (DNA and RNA) (Insam 2001; Kirk et al. 2004), significant progress has been made in their study. The combined use of different molecular techniques based on the small-subunit rRNA has generated important data on the diversity, structure and dynamics of microbial communities (Rastogi and Sani 2011; Rajendhran and Gunasekaran 2008). For example, DNA-based molecular studies have shown that clone libraries constructed from soil can be composed almost entirely of single members of microbial communities (Zhou et al. 2002). In fact, Fierer et al. (2007) reported that archaeal and fungal communities were consistently less even than bacterial communities were. The vast majority of communities are scarce, and a few are the most abundant, suggesting that there are competitive interactions that determine their structures (Zhou et al. 2002), especially in saturated soils, compared to surface soils, where there is no dominant competition between communities. Two of the

characteristics that promote this diversity are the stability of soil moisture in deeper layers, and the high competition for carbon sources that are not as abundant as in surface layers; moreover, it has also been shown that the diversity of microorganisms increases with decreasing soil particle size (Sessitsch et al. 2001).

Microarrays have enabled the analysis of large volumes of data to detect gene expression or find the presence of array sequences in a given experimental sample (Murray et al. 2001; Chang et al. 2008). Thus, different types of microarrays (open reading frame arrays for complete genomes, phylogenetic oligonucleotide arrays, community genome arrays, metagenomic arrays, and functional gene arrays such as GeoChip arrays) have been developed to examine microbial populations either in natural ecosystems or with different degrees of disturbance (Van Nostrand et al. 2011). These techniques represent specific, sensitive and rapid tools for analyzing microbial communities from different environmental samples (He et al. 2012). Using such tools it has been shown that the bacterial community, in terms of diversity, changes depending on the type of soil (considering its pH, nutrient availability, oxygenation, dominant flora, leaf litter decomposition rates, rhizosphere/mycorrhizosphere, etc.) (Prescott and Grayston 2013). Thus, Russo et al. (2012) demonstrated that analysis of *16S rRNA* genes by PhyloChip microarrays showed that the richness of bacteria is greater in clay soil (Acidobacteria group) compared to sandy-loam soil (Proteobacteria class); however, the bacterial classes Actinobacteria, Betaproteobacteria, Clostridia, Bacilli, and Gammaproteobacteria were more abundant in sandy-loam soil.

Davinic et al. (2012) analyzed the bacterial diversity in soil macro- and micro-aggregates, showing that each microenvironment hosts a different bacterial community. Thus, macroaggregates with high carbon content showed abundance of Actinobacteria (excluding the order Rubrobacteriales) and α -Proteobacteria; by contrast, microaggregates showed greater abundance of Rubrobacteriales and a lower amount of organic carbon.

Through pyrosequencing of *16S*- and *18S-rRNA* genes, Bastida et al. (2013) determined that the structure of bacterial and fungal communities showed variations in response to the presence or absence of organic amendments. The number of α -Proteobacteria sequences was higher than that of Actinobacteria sequences in soils with high amendment application. As for fungi, Ascomycota sequences responded positively to restoration.

Beyond the genome and metagenome (the whole genetic material from an environmental sample), analysis of the proteome and metaproteome contributes to our understanding of the microbial biodiversity in the soil/rhizosphere and their functions in aspects related to plant-microorganism molecular dialog, plant nutrition and

nutrient recycling (Badri et al. 2009; Xiong et al. 2013; Renella et al. 2014).

To support the annotation, analysis and distribution of microbial genome and metagenome datasets, the US Department of Energy Joint Genome Institute (DOE JGI) created The Integrated Microbial Genome (IMG) system, which is also open to the scientific community worldwide (Markowitz et al. 2014). The IMG data warehouse integrates genomes from all three domains of life, as well as plasmids, viruses and genome fragments. IMG provides tools for analyzing and reviewing the structural and functional annotations of genomes in a comparative context. Different IMG datamarts provide support for the analysis of publicly available genomes (IMG/W: <http://img.jgi.doe.gov/w>), expert review of genome annotations (IMG/ER: <http://img.jgi.doe.gov/er>) and teaching and training in the area of microbial genome analysis (IMG/EDU: <http://img.jgi.doe.gov/edu>).

According to the IMG platform, in May 2015 there were 8257 sequenced microbial genomes, of which 3404 correspond to bacteria, 199 to archaea, 31 to eukaryota, 1186 to plasmids and 3348 to viruses (Table 1). In total, 98,482,933 genes have been sequenced.

Furthermore, the Community Cyberinfrastructure for Advanced Microbial Ecology Research and Analysis (CAMERA, <http://camera.calit2.net/>) is a database and associated computational infrastructure that provides a single system for depositing, locating, analyzing, visualizing and sharing data about microbial biology through an advanced web-based analysis portal (Sun et al. 2011) which substantially supports any initiative related to environmental microbiology, including microbial-mediated plant nutrition.

Going higher up in the complexity of sequencing projects, metagenomes of microbial communities are derived from complex processes of direct DNA extraction from microorganisms inhabiting ecosystems, with which metagenomic libraries are constructed. Metagenomic libraries contain the entirety of information on the integral genetic capacity of biological systems, including communities composed of a multitude of species, which are easily accessible for other experiments and applications. Figure 1 shows the overall process for analyzing environmental samples either through the construction of libraries or through direct sequencing of total DNA for subsequent assembly using bioinformatics tools (Lasken 2012; Lorenz and Eck 2005).

Metagenomic applications enable us to not only understand the molecular processes that generate biodiversity, but also to recreate them in experimental conditions for detailed study of nutrient uptake and transport processes, to cite one example. Microbial communities in the plant rhizosphere are crucial in the processing of nutrients that the

Table 1 Microbial genomes sequenced, in the process of being sequenced or drafts (Version 4.520 April 2015)

Category	Sequenced genomes	Genomes in the process of sequencing	Drafts	Total
Bacteria	3404	2526	19,941	25,871
Archaea	199	15	318	532
Eukaryota	31	37	122	190
Plasmids	1186	0	0	1186
Viruses	3348	53	487	3888
Genomic fragments	89	0	1103	1192
Total	6937	3228	21,971	32,859

Considering the Integrated Microbial Genomes database (<http://img.jgi.doe.gov/cgi-bin/edu/main.cgi?section=ImgStatsOverview#tabview=tab0>)

Fig. 1 Metagenomic analyses of environmental samples either by direct sequencing of lysed DNA or by constructing metagenomic libraries containing DNA cloned into different vectors

root can absorb and transport. Certain bacteria convert atmospheric nitrogen into ammonia. Some soil organisms recycle nutrients from the remains of plants and animals, while others convert elements such as Fe and Mn into forms that can be used by the plant. Furthermore, no microorganism can convert ammonium to nitrate in isolation, but groups of them can do so efficiently. This demonstrates the potential that microbial communities can have in processes essential for life such as biotransformations in soil (Alarcón et al. 2012).

The applications being developed and those that can only be glimpsed now have immense potential. Therefore, the changes that lie ahead with these advances will place soil science and crop nutrition research in a new era of development. With these technologies, it is possible to know the structure and function of organisms never before discovered or that had not been possible to cultivate. For example, Fierer et al. (2012a) sequenced the metagenomes of samples from cold deserts, hot deserts, forests, grasslands and tundra. The desert communities had higher relative abundances of genes associated with osmoregulation and dormancy, but lower relative abundances of genes associated with nutrient cycling and the catabolism of plant-derived organic compounds. By studying the physiological mechanisms in which the products of these genes are involved, it will be possible to know how microbial communities can survive in environments with limited water and nutrients (Fierer et al. 2012b).

Molecular plant nutrition

Agricultural food production requires the application of fertilizers containing various nutrients, mainly N, P and K. The current availability of plant genome sequences and the development of molecular biology techniques have accelerated the identification of the assimilation pathways of these nutrients and the genes responsible for them. These advances have enabled biotech efforts to be focused on studying the regulation of these pathways and the interaction among them, to make more efficient use of these inputs and have a less negative impact on the environment. To date, 15 genomes of green algae and 185 of terrestrial plants have been completely sequenced or are in the process of being sequenced (<http://www.ncbi.nlm.nih.gov/genome/browse/>). While many of these genomes are not yet complete, they have already proven to be a tool of great value to undertake breeding programs for agronomic traits such as fruit set, grain production and flowering time (Bolger et al. 2014). Figure 2 shows a summary of the genome sequencing of plants of agronomic interest and some model plants since the publication of the first complete genome of a higher plant, *Arabidopsis thaliana*, in

2000 (AGI 2000), to the last released of the cotton genome in May, 2015 (Li et al. 2015). To date, sequence genomes of crop plants include rice (*Oryza sativa*), papaya (*Carica papaya*), maize (*Zea mays*), woodland strawberry (*Fragaria vesca*), tomato (*Solanum lycopersicum*), sugar beet (*Beta vulgaris*), Chinese cabbage (*Brassica rapa*), muskmelon (*Cucumis melo*), cucumber (*Cucumis sativus*), soybean (*Glycine max*), cocoa tree (*Theobroma cacao*), and apple (*Malus domestica*), among others (Schatz et al. 2012; Bolger et al. 2014).

Given the limited sources of fertilizers such as rock phosphate, a nonrenewable resource, and the general cost increases for these inputs, it is necessary to look for alternatives in the biodiversity of the soil itself. This could be achieved through microorganisms that take advantage of these nutrients and make them available to plants, or through the exploration of genotypes that efficiently use water and nutrients. In addition, the increase in sequenced crop plant genomes provides a huge reservoir of information that could be exploited to make better use of manures, fertilizers and bioinoculants. The genomes of the model plants *Lotus japonicus* (Sato et al. 2008) and *Medicago truncatula* (Li et al. 2012), as well as of symbiotic microorganisms such as *Rhizobium* spp. (Servín-Garcidueñas et al. 2012), offer a window into the study and application of research on symbiotic interactions for more efficient use of fertilizer inputs and plant growth regulation. The following section discusses some of the most important advances concerning the metabolism of the major macronutrients, namely, N, P and K, on the basis of genomic and biochemical studies, as well as on microbially mediated plant nutrient transformations.

Nitrogen (N)

Plants can absorb and use various forms of N present in the soil, the most important being ammonium (NH_4^+) and nitrate (NO_3^-), and in legumes, N can be obtained through symbiotic fixation processes. Some species show a strong preference for one ionic form over the other, although in sustainable agriculture it is necessary to look for an efficient use of these nitrogen sources and a balance between them (Wang et al. 2014a).

Plants have developed various mechanisms to regulate N metabolism in response to N availability and environmental conditions, and various enzymes are involved in the absorption, reduction, and assimilation of N sources. In higher plants, three families of transport proteins determine NO_3^- transport. The first family comprised of one of low affinity (LATS or low-affinity nitrate transport system), represented by the NRT1/PTR family of transporters that act in soils or culture media with high nitrate concentrations. The second and third families are composed of high

Fig. 2 Genomes of model and agriculturally important plants sequenced or in the process of being sequenced according to NCBI (<http://www.ncbi.nlm.nih.gov/genome/browse/>). Column colors on the left of the DNA helix represent the underlying techniques and sequencing strategies used, while that on the right depicts the approximate timeline of when genomes were sequenced. The distinction between pure NGS and hybrid (Sanger +454/Illumina/SOLiD) sequencing is sometimes arbitrary, as many genome projects rely on previously generated Sanger sequences. NGS next generation sequencing

affinity (HATS or high-affinity nitrate transport system) constituted by the NRT2 and NRT3 (NAR) families of transporters which function when the available nitrate in the soil or growth medium is limited. The NRT1 family comprises 53 members, of which only nine have been characterized at the functional level and are predominantly low-affinity transporters; the NRT2 family contains seven members of high-affinity transporters; and there are two members of the NRT3 (NAR2) family involved in high-affinity transport (Krapp et al. 2014).

Regarding ammonium (NH_4^+), its transporters are called AMT, and they are essential elements of the uptake pathway and homeostasis of NH_4^+ in the root, since the uptake of this ion might cause toxicity, and it can be produced through other catabolic pathways in the cell. As ammonium homeostasis mechanisms, plant genomes contain several AMT genes that encode for transporters that differ in their biochemical properties, location and in their regulation at the transcriptional level (Loqué and von Wirén 2004). In *Arabidopsis thaliana*, AMT genes make up

a family of six members (two subfamilies) that transport NH_4^+ in high-affinity systems (Krapp et al. 2014).

In a recent review, von Wittgenstein et al. (2014) highlighted that nitrogen uptake, reallocation within the plant, and between subcellular compartments involved ammonium, nitrate and peptide transporters. In angiosperms, each of the two families of NH_4^+ transporters (AMT1 and AMT2) comprised of five members on average in angiosperms, while NO_3^- transporters also form two discrete families (NRT1 and NRT2), and angiosperm genomes encode only four NRT2s, on average. NRT1s share an evolutionary history with peptide transporters (PTRs). The NRT1/PTR family in land plants usually has more than 50 members and contains members with distinct activities, such as glucosinolate and abscisic acid transport. Identification and characterization of these gene families include the crop plants rice, sorghum, maize, soybean, papaya, cucumber, cassava, peach and grapevine.

Transcriptomic analyses of plant responses to nitrate have revealed a linkage between N and S, and between N

and Fe (Wang et al. 2003). Nitrate applications to N-deficient plants induce the expression of the *nicotianamine synthase* gene, which is involved in Fe uptake, transport and homeostasis in plants (Pich et al. 2001; García et al. 2010). Iron is required for the activity of many of the enzymes involved in the reduction and assimilation of nitrogen, including nitrate reductase, nitrite reductase and ferredoxin. Therefore, nitrate-induced nicotianamine synthesis probably occurs to facilitate Fe transport to support the synthesis of proteins for nitrate assimilation (Ohkama-Ohtsu and Wasaki 2010). The gene expression involved in hormone synthesis and perception is also affected by nitrate, and it has been demonstrated that this stimulates cytokinin biosynthesis (Takei et al. 2001). Two more hormones, auxins and abscisic acid, are involved in nitrate-induced lateral root growth inhibition (Liu et al. 2009).

Recent advances in the area of biofertilizers, specifically those related to symbiotic N-fixing bacteria of the genus *Rhizobium*, and to free-living rhizobacteria of the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Serratia*, and *Streptomyces*, show that it is possible to make efficient use of this resource without applying excessive concentrations of mineral fertilizers rich in N (Reddy 2014). In the case of arbuscular mycorrhizal (AM) fungi, specific ammonium transporters have been detected in the fungal mycelium, which enhance N uptake and use by the host plant (Pérez-Tienda et al. 2011, 2014).

Production and application of N fertilizers consume huge amounts of energy, and their excess use is detrimental to the environment. Improving N use efficiency (NUE) is therefore crucial, and represents a significant challenge for sustainable agriculture. For economically and environmentally friendly use of valuable N resources, developing high NUE cultivars is more challenging than targeting N applications as part of integrated nutrient management (FAO 2006). Complex multigene traits for NUE include the integration of genotypic and environmental conditions, particularly N supply. The proper evaluation of plant NUE to identify the main limiting factors for maximizing NUE has to be considered for crop improvement. The most important aspect of the different NUE components is the N requirement for producing the highest potential yield (Xu et al. 2012).

Even though N is the most studied macronutrient in plant metabolism, further studies are required of its uptake, transport and mobilization in plants. Identification and characterization of molecular mechanisms involved in such processes, as well as learning in greater detail the mechanisms used by soil microorganisms in the symbiotic fixation of this element and the exchange of cell signals in symbiotic relationships with host plants, remain to be elucidated (Santi et al. 2013; Yadav et al. 2015). Thanks to the most recent genomic and metagenomic projects, our

understanding of the players and pathways of the global nitrogen cycle has advanced substantially over the last few years with discoveries of several new groups of organisms and new types of metabolism. Recently discovered processes add new functionality to the nitrogen cycle; these functions include denitrification and other dissimilatory nitrogen transformations in eukaryotes, anaerobic ammonium oxidation, and anaerobic methane oxidation with nitrite. The ecophysiology of the novel organisms and their interactions with classical types of nitrogen metabolism are important for understanding the nitrogen cycle and its tight links to the cycling of carbon, in the past, the present and the future (Thamdrup 2012).

Phosphorus (P)

Modern agriculture depends on the phosphorus supply that mostly comes from mined rock phosphate, which is a nonrenewable resource, and current global reserves may be depleted within a period of 50–100 years (Cordell and White 2011). While demand for this nutrient is projected to increase, peak production is predicted to occur before 2035, while the quality of rock phosphate (Pi) is decreasing and processing costs are rising (Cordell et al. 2009). It is therefore necessary to undertake strategies that make more efficient use of this resource, through sustainable technologies such as recycling and the use of P-solubilizing microorganisms.

Levels of Pi are suboptimal in most natural and agricultural ecosystems, and when Pi is applied as fertilizer in soils, it is rapidly immobilized owing to fixation and microbial activity (López-Arredondo et al. 2014). When Pi is supplied as fertilizer, it can be also immobilized due to its high reactivity with cations such as Ca and Mg in calcareous soils or Al and Fe in acidic soils. The abundance of soil microorganisms also affects Pi nutrition in a complex manner: while AM fungi and certain plant growth-promoting rhizobacteria may enhance plant Pi acquisition, many other microorganisms have adverse effects because of their competition with roots for Pi uptake and because their activities convert Pi into organic forms that are not readily available for plant uptake (Hinsinger 2001; Vance et al. 2003). Even under a well-designed Pi fertilization scheme, plant roots acquire no more than 30 % of the applied Pi. This situation has led to the excessive application of fertilizers, contributing to the nutrient enrichment of water bodies that results in eutrophication and toxic algal blooms (Smith and Schindler 2009). Ironically, this phenomenon is leading to the extinction of hundreds of species. The unrestrained exploitation of rock phosphate, and our dependence on it for agriculture may lead to an unsafe situation in which Pi might become a commodity to be fought over, whilst at the same time, health and

environmental experts are likely to recommend reductions in its use (Ferro et al. 2015).

Recent advances in our understanding of mechanisms by which wild and cultivated plant species adapt to low-phosphate conditions and the use of alternative bacterial pathways for P metabolism have resulted in the design of more effective breeding and genetic engineering strategies to produce highly Pi-efficient crops, optimize fertilizer use, and reach agricultural sustainability with a lower environmental cost (López-Arredondo et al. 2014). To develop low-Pi-tolerant crops, current research advances concerning the complex network of plant responses to Pi deficiency may allow the implementation of biotechnological strategies that take advantage of the genes involved in phosphate uptake, remobilization, and metabolism.

Plants acquire phosphorus from the soil solution as phosphate anion (Pi). It is the least mobile element in plants and soil, in comparison with other macronutrients. At the molecular level, the Pi ion is absorbed by the roots and translocated to other organs through phosphate transporters (PiT). To date, numerous proteins that encode PiT transporters in different species, including *Arabidopsis*, cereals, legumes and nightshades, have been found. The *Arabidopsis* genome contains 19 *PiT* genes clustered into four families: *PHT1* to *PHT4* (Wang et al. 2014b). The *PHT1* family of proteins comprised of high-affinity Pi/H⁺ symporters localized in the plasma membrane and their main function is to acquire Pi from the rhizosphere since most of them are expressed in the epidermis of root cells, whereas the other *PHT* families are located in other endomembrane systems. For example, *PHT2* transporters are only localized in chloroplasts (Versaw and Harrison 2002), *PHT3* ones in mitochondria (Poirier and Bucher 2002), and *PHT4* members in non-photosynthetic plastids and in the Golgi apparatus (Guo et al. 2008). In addition to Pi, PiT transporters can absorb phosphite (Phi), an analog of Pi. Although the uptake and mobility of Phi and Pi are similar, there is no evidence showing that plants can utilize Phi as their sole source of phosphorus. Furthermore, it has been demonstrated that Phi interferes in many plant responses to Pi deficiency. For example, Phi inhibits root growth and the root/shoot ratio in Pi-deficient media but not in sufficient conditions of this ion. Interestingly, Estrada-Ortiz et al. (2013) showed that adding Phi to the nutrient solution used for growing strawberries improves some fruit quality characteristics when there is sufficient Pi, and it has been postulated that Phi could activate defense mechanisms against pathogen attack. In fact, Oyarburo et al. (2015) recommend the use of Phi as an agent to activate defense, cellular signal transduction and quality improvement mechanisms in certain conditions and for certain crops. The only way that plants can use Phi as a P-source is through the expression of a bacterial phosphite

dehydrogenase, as has been reported for *Arabidopsis thaliana* and *Nicotiana tabacum* (López-Arredondo and Herrera-Estrella 2012), which opens up new opportunities to use Phi as a P-source in P nutrition approaches.

Phosphate is precipitated as orthophosphate or adsorbed by Fe and Al in acid soils or by Ca or Mg in basic soils (Vance 2001; López-Arredondo et al. 2014). Phosphorus-solubilizing bacteria play a very important role in phosphate nutrition by enhancing its availability to plants through the release of this element from organic and inorganic soils (Mohammadi 2012). The main mechanism that exists in soil to solubilize phosphate is through lowering the pH by the production of organic acids by microorganisms and mineralization of organic P by means of acid phosphatase enzymes. The use of P-solubilizing bacteria as inoculants increases P uptake from the soil. These bacteria also enable the use of rock phosphate for crop production (Nobandegani et al. 2015). In this regard, Singh and Satyanarayana (2011) reviewed the importance of phytases and the microorganisms that produce them, in the use of organic phytate sources. Among the most promising P-solubilizing microorganisms are bacterial strains of the genera *Pseudomonas*, *Bacillus* and *Rhizobium* and fungi of the genera *Galactomyces* and *Penicillium*. According to Zaidi et al. (2009), among the diverse and abundant microbes inhabiting the rhizosphere, those that possess the ability to solubilize phosphate represent an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants. Through conventional methodologies for morphological and functional characterization of microorganisms and analysis of 18S *rRNA* gene sequences, Wu et al. (2012a) isolated the fungus *Galactomyces geotrichum* strain P14 (P14), which showed ample ability to release phosphate and solubilize rock phosphate, implying great potential for use as a biofertilizer to enhance soil fertility and promote plant growth. Subsequently, Chai et al. (2011) isolated a fungus identified as *Penicillium* sp. strain PSM11-5, which showed high phosphate-solubilizing ability in media with pH values ranging from 5.0 to 8.0. Vassileva et al. (2010) observed that a single phosphate-solubilizing organism can show a wide range of metabolic abilities in different environments. When grown in bioreactors, these organisms can produce several types of enzymes, siderophores and plant growth regulators. The introduction of these biotech products in the soil–plant system improves plant growth, soil properties, soil biological activity, and plant defense mechanisms.

Another biological mechanism is represented by mycorrhizal symbiosis. In this mechanism, the establishment of AM fungi in the root of a host plant results in the promotion of plant growth by direct effect of improving the uptake and utilization of essential nutrients in plant

metabolism (Chen et al. 2013; Ouziad et al. 2006; Pérez-Tienda et al. 2011, 2014).

Various research groups worldwide have analyzed global changes in plants in response to P, either through transcriptomic studies (Wasaki et al. 2006), metabolomic studies (Huang et al. 2008) or integrated ones [transcriptomics, proteomics and metabolomics; (Morcuende et al. 2007)]. Phosphorus deficiency quickly causes the induction of high-affinity P transporters and acidic phosphatases, as well as glycolysis, possibly as a mechanism to produce organic acids which allow the recycling of P from phosphorylated intermediate compounds. Unlike the general repression of amino acid synthesis caused by N deficiency, P deficiency only induces aromatic amino acid synthesis, but represses the synthesis of nucleic acids and proteins and the gene expression involved in photosynthesis (Ohkama-Ohtsu and Wasaki 2010). Mission et al. (2005) also demonstrated the interaction between P and Fe. It has thus been possible to detect genes encoding phosphate transporters in plants that establish symbiosis with AM fungi (Chen et al. 2013; Ouziad et al. 2006).

Recently, Becquer et al. (2014) described the journey of P from the soil to the plant through trophic relationships and ectomycorrhizal association to understand the ecological and molecular mechanisms responsible for transferring Pi and improving plant P nutrition. Ectomycorrhizal fungi improve nutrient status of plants, as they are able to release organic anions or phosphatases to mobilize unavailable P (Louche et al. 2010; Plassard and Dell 2010). Furthermore, bacteria play a major role in the mineralization of P through trophic relationships as they can produce phytases to degrade phytate, the main form of soil organic P. Bacteria are also more effective than other microorganisms or plants at immobilizing free Pi. Interestingly, free Pi may be taken up by ectomycorrhizal fungi by specific phosphate transporters and transferred to the plant by mechanisms not yet been identified (Becquer et al. 2014).

Despite the numerous studies that have been conducted on these microorganisms and their multifunctional properties, the development of commercial bioinoculants is still far from realization. In fact, direct inoculation of free phosphate-solubilizing bacteria (PSB) into soil does not easily maintain cell survival around plants roots because they are susceptible to a variety of environmental variations (Wu et al. 2012b). Variability in the effect of PSB inoculants applied to crops is largely due to the relative effectiveness of the bacterial strain in the inoculant formulation, which can determine the success or failure of a biological agent.

Interestingly, immobilization of bacterial cells has been widely used in agriculture to achieve a protective structure or capsule allowing immobilization, protection, release and

functionalization of active ingredients (Schoebitz et al. 2013). Therefore, current progress in studies exploring functional diversity, the ability to colonize the rhizosphere, mode of action and applications in agriculture and industry may facilitate their use as reliable components in sustainable agricultural systems of the future.

Potassium (K)

Over 90 % of potassium in soil exists in the form of silicate minerals (microcline, muscovite, orthoclase biotite and feldspars, among others) (Meena et al. 2014). These minerals can be weathered through protonation processes but also form complexes with organic acids, and it has been shown that some acidic polysaccharides of microbial origin may also have some role in these processes (Ehrlich 2002).

The average K reserves in soil are abundant, although most of it is not available to the plant. Therefore, crops have to be supplied with soluble K and the demand for it is increasing every year. Organic exudates of some bacteria and plant roots play a key role in the release of K contained in minerals, which is why the search for genotypes that show better mechanisms for accessing the K contained in these rock materials is one of the main breeding approaches in sustainable agriculture. Potassium deficiency affects the flow of photosynthates to sink organs, which causes sugars to build up in the leaf and they, in turn, reduce yields and crop quality. Because of its pivotal role in turgor, primary metabolite generation and long-distance transport processes, K is a key component in mechanisms providing tolerance to drought, salinity, high brightness and cold, as well as to pathogens and pest insects. Despite its importance in these and other vital processes, there has not been strong interest in breeding to increase uptake and efficient use of this element, which requires rethinking the study objectives of various working groups in the world and prioritizing research on K as a key macronutrient in soil fertility and food security (Zörb et al. 2014).

In plants, K membrane transport is mediated by K channels and second by K transporters (Gierth and Mäser 2007). In the model plant *Arabidopsis thaliana*, there are five families of genes involved in uptake, transport and mobilization of K from the soil to the fruit and within the plant itself: two families of different channels (15 genes); two families of transporters (14 genes) and a family of antiporters (six genes). K⁺ channels are by far the best characterized enzymes in terms of regulation of ionic input and output, second messengers and transport (Sharma et al. 2013). However, little is known about permease-type transporters and antiporters (Gómez-Porrás et al. 2012). Recently, Véry et al. (2014) performed a comparison of some of these family systems between species, including *Arabidopsis*, rice and grapevine, giving insights into the

structure of their members and on functional properties and their roles in K metabolism.

In plants, K deficiencies facilitate the entry and spread of pathogens and insects (Amtmann et al. 2008). Microarray experiments revealed strong repression of N transporters in K-deficient plants, which is quickly counteracted to supply sufficient K to the plants. Crop yield in conditions of K deficiency and attack by pathogens and insects is differentially affected by the degree of synergistic and antagonistic effects which occur in the plant in response to this kind of stress (Armengaud et al. 2004), and it has been discovered that some symbiotic organisms can contribute to potassium buildup in certain host plants (Basak and Biswas 2010).

Some species of microorganisms, including *Pseudomonas* spp., *Burkholderia* spp., *Acidithiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Bacillus edaphicus* and *Bacillus megaterium*, have the ability to release K from materials containing this element by exudation of organic acids (Zörb et al. 2014). These acids directly dissolve the K contained in rock minerals or chelate the primary ions contained in the minerals to release K (Basak and Biswas 2009). As a result, inoculation with K-solubilizing microorganisms together with the application of minerals rich in K are strategies that are receiving great interest worldwide, as evidenced by the work of Sheng (2005), Han et al. (2006) and Meena et al. (2014) in different crop plants. Exudates produced by these organisms can increase the release of potassium from clay minerals and K solubility by up to 60 % (Han et al. 2006; Basak and Biswas 2009; Abou-el-Seoud and Abdel-Megeed 2012). El-Hadad et al. (2011) reported that biofertilizers formulated with *Paenibacillus polymyxa* (N-fixing bacteria), *Bacillus megaterium* (P-solubilizing bacteria), and *Bacillus circulans* (K-solubilizing bacteria) have nematicidal activity against *Meloidogyne incognita*, demonstrating that these bacteria not only participate in the mobilization of the major macronutrients (N, P and K), but are also involved in the biological control of nematodes such as *M. incognita*. However, there is a need for more field studies, because what has been done until now has only been carried out in laboratory and greenhouse conditions.

Meena et al. (2014) highlighted the pivotal role played by potassium-solubilizing microorganisms (KSM) in K plant nutrition. A large number of saprophytic bacteria (including *Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *Acidithiobacillus ferrooxidans*, and *Paenibacillus* spp.) and fungal strains (*Aspergillus* spp. and *Aspergillus terreus*) mediate K-solubilization in soil system. The main mechanisms of KSM are acidolysis, chelation, exchange reactions, complexolysis and production of organic acid. Once K has been solubilized by KSM, the plant can easily take it up for growth and development.

Here, one of the major challenges to support sustainable agriculture is to identify and characterize indigenous K-solubilizing microbes to develop efficient microbial consortia for solubilization of K in soil, thus enhancing plant growth and crop yields.

Just recently, on the basis of 16S ribosomal DNA (16S rDNA) sequencing, Meena et al. (2015) identified 12 K-solubilizing rhizobacteria (KSR). Among the assessed 12 KSR, *A. tumefaciens* OPVS 11 and *R. pusense* OPVS6 occurred at the highest K-solubilizing frequency. These strains demonstrated significant reduction in media pH and increased K release with incubation period under both waste muscovite and biotite as a sole source of insoluble K mineral.

Scope and limitations of genomic approaches

It was about 15 years ago that the first genome sequencing of a higher plant, *Arabidopsis thaliana*, was completed, and about 13 years ago that the sequenced genome of a crop plant, rice, was published. From that time on, the number of sequenced genomes of crop plants has grown steadily. At present, 63 % of the sequenced plant genomes belong to crop species (Michael and VanBuren 2015). The rate at which these genomes are being made publicly available is due to the improvement in sequencing technologies, both in terms of time and cost. Modern technologies allow sequencing of multiple genotypes of a crop at a reasonable cost. Although most of the published genomes are not complete, the data they provide are very valuable for understanding important agronomic traits such as fruit set, grain production, and flowering times.

Genomic tools have enormous potential to expand the applications of the metabolic capabilities of microorganisms in plant nutrition strategies, as well as to determine in greater detail the mechanisms that plants have for better nutrient utilization. Although most are still costly, these tools can stimulate the development of innovative technologies for plant nutrition. While some of these technologies are still under development and need to be optimized, their application in certain areas has already proven to be successful, which has led to greater scientific and industrial interest in the identification of key molecules, metabolic pathways and applications in crop nutrition.

In contrast to the tremendous advances in throughput, assembling sequencing reads remains a substantial endeavor, much greater than the sequencing efforts alone would suggest (Salzberg et al. 2012). Large complex plant genomes remain a particularly difficult challenge for de novo assembly for a variety of biological, computational and biomolecular reasons addressed by Schatz et al. (2012).

Therefore, a general interdisciplinary effort must be made to develop efficient technologies to assemble these genomes accurately, make better use of the potentially highly fragmented assemblies and perform these applications at the lowest cost.

The emergence of metagenomics has the potential to revolutionize plant nutrition since it provides direct access to microbial communities that inhabit environments (optimal or limiting), in their entire complexity and regardless of the feasibility of being cultivated. Isolation and characterization of functional genes as well as sequencing of complete metagenomes have the potential to lead to the discovery of new metabolic activities and generate valuable information for the best use of manures and fertilizers under a sustainable development perspective.

Nevertheless, many of the available metagenomic techniques and protocols have their own limitations (Rajendhran and Gunasekaran 2008). One of the constraints on these methods is the complexity of the sample per se and the pollutants present in it, including humus, organic contaminants and heavy metals, among others, that impede its processing (Rajesh et al. 2012). Another common obstacle is the lysis of the full sample and the removal of substances that interfere with DNA extraction (Dua et al. 2002). Lack of knowledge of local and global diversity of microbial communities and populations with symbiotic ability with cultivated plants is also a limitation. In addition, there is little exchange of information and standards among microbial ecology laboratories to enable comparative analyses.

Functional analyses of environmental genomic libraries are not designed to identify genes involved in uptake, transport, and nutrient metabolism processes (Mirete et al. 2007; Guazzaroni et al. 2013). While these functions could be studied using microarrays, the nature of the samples and the limited availability of universal controls impose further complications for understanding the intensity of the detected signals and their correlation with specific species. Furthermore, microarray-based analysis is only qualitative because it only detects the presence or absence of a particular microbial group.

Other methodologies involving gold nanoparticles, quantum dots and magnetic particles are being introduced to the market and offer promising strategies for in situ analysis of microbial communities. However, to date, none of them has been optimized to make direct specific measurements in the environment (Rincon-Florez et al. 2013).

Despite the enormous progress made, metagenomic approaches cannot yet be widely applied in plant nutrition, and the tools used have achieved few concrete results. One of the reasons that may explain this is the fact that functional analyses of metagenomic libraries are limited by the lack of appropriate analysis tools and the insufficiency of

available host systems. Nor are there any reports of obligate or facultative anaerobic hosts being used for analyzing metagenomes in oxygen-deprived conditions, which represents one of the major challenges given the importance of microbial metabolic processes in anoxic conditions. Hence, one of the most pressing priorities is to develop host and expression systems in these oxygen-deprived conditions. Furthermore, large-scale genome sequencing projects in agricultural environments and analysis of the information collected take years, thereby delaying efforts to apply the information generated in crop nutrition initiatives.

One of the major challenges to come involves the amount of data generated through metagenomic projects. Deposits of microorganism sequences are increasing every year, and they are expected to grow exponentially as sequencing technologies improve. However, massive sequencing alone is not capable of defining specific metabolic functions, so it must be coupled to advances in basic microbiology, biochemistry, functional genomics and bioinformatics. New computational tools to be generated in the future will enable defining the functions of new proteins discovered in metagenomic efforts, which may benefit plant nutrition approaches that are being developed.

The good news is that there is growing interest in carrying out more in-depth analyses. For example, Edwards et al. (2013) demonstrated that new generations of life scientists are able to develop strategies to generate and take advantage of information in the new era of sequencing and genomic sciences.

The genomic sciences are faced with two additional challenges, which are partially opposed. On the one hand, the dynamics of microbial communities in response to stressful environments is complex, and the biodiversity in oligotrophic environments such as soils, as well as the number of new genes, is much greater than first predicted. However, metagenomic data only give a partial picture of this diversity and the contribution of particular low-abundant taxa is not reliable. The isolation of individual cells and their sequencing could provide more information about the biological processes that take place in microbial cells that develop in these environments (Lasken 2012).

Together with deep phenotyping platforms, which promise to overcome some technical bottlenecks, we can expect an even faster elucidation of numerous gene functions, but we will be challenged by the sheer magnitude of data available.

Beyond the current advances in plant genomics and microbial metagenomics, the advent of next generation sequencing (NGS) technology has resulted in an urgent need for novel tools to analyze those high-throughput datasets, as well as for young scientists able to develop new computer and engineering strategies to take advantage of changing whole genome and metagenome analyses.

Progress in the field of NGS and omics, beyond the classical genomics, transcriptomics, proteomics and metabolomics, has led to the establishment of new molecular concepts such as exomics (Franzosa et al. 2015; Kahl 2015; Kawalia et al. 2015). Exome sequencing represents a significant tool for assessing natural evolution in plants, identifying gene pools involved in symbiotic and other co-existential systems, and improving crop production, as exons assist in interpretation of allelic variation with respect to their phenotype (Hashmi et al. 2015). These technologies are used in exploring the biodiversity of crops, thereby providing better understanding of genomic resources for crop wild relatives so that they can be used in breeding, allele identification and introgression. Draft genomes from wild relatives of some crop species are currently available, but more wild species are needed for crop improvement programs (Michael and VanBuren 2015).

These issues represent major challenges for the genomic sciences that will have to be addressed under interdisciplinary approaches in the future.

Conclusions

Soil science has advanced greatly thanks to the progress made by molecular biology, especially genome sequencing, to understand and characterize this complex system, both at the level of microorganisms that inhabit it and the plants that thrive on it. This has enabled the development of new fields of knowledge such as metagenomics, molecular microbiology and molecular plant nutrition. It has thus been possible to learn in detail how microorganisms intervene in plant nutrition and growth, and how they absorb, transport and take advantage of nutrients. The challenge for the sustainability of agriculture and food security lies in effectively utilizing soil biodiversity through better knowledge of the organisms that inhabit this complex system. In addition, plant mechanisms that determine better use of these inputs need to be further investigated, especially since some mineral fertilizer sources on the planet are prone to run out, become increasingly expensive, and negatively impact the environment. Molecular biology and metagenomics are tools that are enabling us to understand these soil resources and to find the best way to take advantage of them for the development of new plant nutrition approaches.

While some genomic and metagenomic disciplines are in development, all of them have the potential to generate unprecedented information on the nature, regulation and evolution of metabolic pathways of plant–microorganism interactions that take place in different environments. This information constitutes the first step towards implementing

effective strategies for efficient nutrient use by plants, and thus these approaches are emerging as solid pillars of research for the years to come. Additionally, a growing need can be seen for scientists with the ability to analyze, interpret and apply this knowledge under biotechnological innovation approaches.

Author contribution statement Authors contributed equally to the paper.

Acknowledgments The Colegio de Postgraduados Priority Research Line No. 5 in Microbial, Plant and Animal Biotechnology supported this work. The authors are very thankful to the Line of Generation and Application of Knowledge No. 1 from the Agri-food Sustainable Innovation MSc Program from the Colegio de Postgraduados Campus Córdoba (Mexico).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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