

Implication of Rhizosphere Acidification in Nutrient Uptake by Plants: Cases of Potassium (K), Phosphorus (P), and Iron (Fe)

Hayet Houmani, Mokded Rabhi, Chedly Abdelly, and Ahmed Debez

Abstract The rhizosphere represents the interface between soil and roots. In this zone, many interactions between plant roots and the soil solution occur and result in important modifications of the physicochemical properties in this area. Plants require large amounts of nutrients to assure maximum growth and development. The absorption of excess nutrients by roots leads to many changes in the rhizosphere such as the acidification phenomenon which is known to be a continuous process in many soils through the world. It is due to the extrusion of protons at the root plasma membrane in favor of cation influx, a principal way used by plants to remove nutrients from the soil solution. Rhizosphere acidification plays a crucial role in nutrient acquisition by plants and is attributed to the activity of H⁺ATPase pumps located at the plasmalemma. The implication of proton release in nutrient acquisition by plants was proved under low nutrient availability. Thus, an increase of the H⁺ATPase activity was noted under a deficiency in many essential nutrients. Due to new advances in molecular biology, the role of H⁺ATPases in nutrient uptake by plants is more elucidated and many genes encoding these pumps are identified. In the present chapter, we summarize information gained on the role of rhizosphere acidification in the uptake of two essential macronutrients and a key micronutrient, respectively, potassium (K), phosphorus (P), and iron (Fe), especially under deficiency conditions and we describe the recent findings related to H⁺ATPases that drive the acidification process.

Keywords Acidification • H⁺ATPase • Iron (Fe) • Nutrient uptake • Nutrient deficiency • Rhizosphere • Proton release • Potassium (K) • Phosphorus (P)

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1 Introduction

Plants require nutrients for their growth and development. These elements are classified into groups. The first, macronutrients, mainly consist of potassium, magnesium, nitrogen, calcium, and phosphorus. The second class is termed micronutrients and includes at least manganese, boron, copper, iron, molybdenum, and zinc. Plants have evolved different mechanisms to acquire such elements from the rhizosphere. The rhizosphere is defined as the critical zone of interaction among three components: plants, soils, and microorganisms (Zhang et al. 2010; Shen et al. 2011) and is considered a key interaction zone between plants and soils (Shen et al. 2013). Therefore many interactions occur between roots and the rhizosphere at the plasma membrane site because plant biological activity can affect the chemistry of the rhizosphere. Indeed, due to their activities, roots are able to modify notably the physicochemical properties of the rhizosphere via the exudation of organic compounds or the release of H^+ protons. Roots, the first organs in direct contact with the rhizosphere, are able to react with their environment and thus can alter the biogeochemistry of the rhizosphere (Schreiber et al. 2011; de Kroon et al. 2012; Postma and Lynch 2012; Hinsinger et al. 2003, 2005, 2009). This plays a key role in enhancing the bioavailability of nutrients such as P (Hinsinger 2001) via the release of proton into the external medium in order to acidify the rhizosphere (Neumann and Römheld 2002; Zhang et al. 2010; Hinsinger et al. 2009; Marschner 2012). As described previously by Marschner (1998), the processes that occur in the rhizosphere including the changes in pH or exudates released by roots play an important role in nutrient acquisition by plants. Rhizosphere acidification is a key mechanism for plant mineral nutrition (Palmgren 2001) because it is responsible for the plasma membrane proton motive force and leads to the solubility of nutrients. Recently, Fujii (2014) mentioned that soil acidification is considered an adaptive trait used by trees to uptake nutrients from the soil. Moreover, the form of nutrient taken up by roots significantly influences the rhizosphere properties. We can cite here the example of nitrogen. If this nutrient is supplied in ammonium form, an acidification of the rhizosphere occurs via the release of protons into the external medium (Taylor and Bloom 1998; Hinsinger et al. 2003). Generally, the uptake of an element is accompanied by the extrusion of a proton in the case of cations, or OH^- in the case of anions. It is known that dissolution of some minerals such as calcium (Ca), iron (Fe), and aluminum (Al) vary greatly with the size of particles and with the soil pH (Pierzynski et al. 2005; Oelkers and Valsami-Jones 2008). For nitrate (NO_3^-) uptake from the soil, an active transport coupled to an H^+ electrochemical gradient generated by the activity of PM H^+ -ATPases across the root plasma membrane (Miller and Aldrich 1996; Forde 2000) takes place in the epidermal and cortical cells.

It was very well established that nutrient uptake from the soil is achieved by cation exchange, where protons (H^+) are pumped by root hairs into the soil. The proton release displaces cations attached to negatively charged soil particles and thereby makes the cations available for uptake by the roots. Because macronutrients are consumed in larger quantities and generally an excess uptake of cation over

anion occurs, this leads to H^+ release into the external medium (Loss et al. 1993; Tang et al. 1997). In fact, when plants absorb high amounts of cations, this is positively correlated with H^+ excretion in each rooting zone. The continuous release of H^+ leads to a decrease of the pH around the roots and results in the acidification of the rhizosphere which is a determinant for cation driving and uptake by plants. Thus, the rhizosphere can be acidified directly under excess cation uptake over anion due to the release of protons from the roots (McLay et al. 1997). This process is related to the activity of hydrogen pumps, H^+ -ATPases at the root plasmalemma, and plays an important role in cation acquisition by plants. It was suggested that the improvement of nutrient uptake and use was often ascribed to rhizosphere acidification.

Nutrients can also influence the uptake of each other via feedback and thus affect plant nutritional status and consequently, a modification of the rhizosphere properties is inevitable (Zhang et al. 2010). Depending on the form of N supply, the uptake of cations and anions is modulated and can affect the pH of the rhizosphere (Marschner 2012). In this context, Shen et al. (2013) showed that localized application of ammonium combined with a superphosphate notably enhanced the productivity of Chinese crops grown in calcareous soil and attributed this effect to the role of ammonium uptake in promoting proton release by roots resulting in a decrease of rhizosphere pH and leading to an increase in the bioavailability of phosphates as documented by Jing et al. (2010, 2012). Given the importance of rhizosphere acidification, many practices have been developed to increase such a process in order to improve nutrient uptake by plants via the manipulation of rhizosphere acidification (Zhang et al. 2010; Chen et al. 2011). Rhizosphere acidification, the principal engine of nutrient uptake for plants, can be stimulated using fertilization management or via screening genotypes with high rhizosphere acidification capacity (Shen et al. 2013). It has been suggested by Guo et al. (2010) that the excessive utilization of nitrogen fertilizer in Chinese intensive agriculture promotes soil acidification in the long term. Thus, a new practice used by agriculture to improve nutrient uptake is the intercropping system, which is defined as an association of genotype with high acidification capacity with other species less efficient in this process. In China, in intercropping systems, optimization of crop combination and nutrient management via a better understanding of the interactions that occur in the rhizosphere is necessary to improve sustainable crop production characterized by a high yield and high nutrient use efficiency (Zhang et al. 2010, 2012). It has been demonstrated by Li et al. (2004) that during the association of chickpea and wheat plants supplied with organic P, the first species improved P nutrition of the second one due to the increase of acid phosphatase activity and to the rhizosphere acidification. Such findings suggested that chickpea released high amounts of protons which are beneficial for both chickpea and wheat having a tangled roots (Li et al. 2007). According to Li et al. (2008a, b), the coculture of wheat and common bean was beneficial in terms of yield productivity and proton release rate which showed a significant increase by such kind of intercropping crops as compared to legumes cultivated individually.

The acidification process has received more attention in the last decade and a multitude of methods are used to study the processes of rhizosphere acidification.

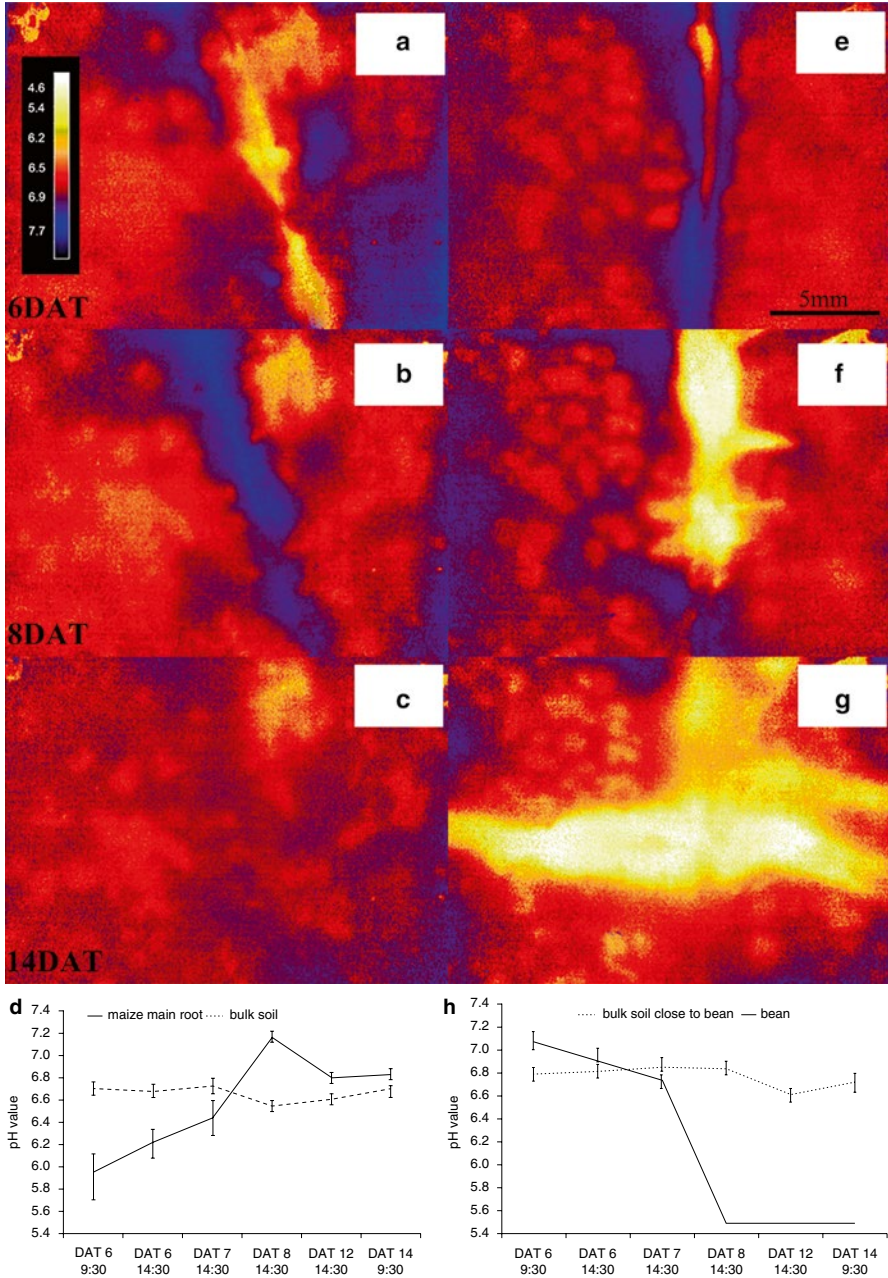


Fig. 1 Use of optodes to measure the dynamics overtime of pH for the rhizosphere and bulk soil of roots of maize (a–d) or bean (e–h) species (a–c, e–g) revealed the pH maps of the respective region of interaction rhizosphere root system (ROI) at a scale pH ranging from 4.6 to 7 at different plant replicates for each species (DAT), DAT6 (a, e), DAT 8 (b, f), and DAT 14 (c, g), respectively. (d, h) show the evolution of the mean pH value within the ROI at the root surface of maize (d) and bean (h) over time growing separately, in rhizotrons in a climate chamber. (Faget et al. 2013)

The pH evaluation can be performed using the newly developed planar optode techniques (Blossfeld and Gansert 2012; Blossfeld 2013). Recently, Faget et al. (2013) found by combination of fluorescence with optode techniques, clear dynamic changes in the pH of the rhizosphere of maize and bean (Fig. 1). A new technique developed by Rudolph et al. (2013) consisting of a spatiotemporal mapping of local soil pH changes induced by lupin and soft-rush was efficient in detecting the acidification of the rhizosphere by these two species.

The acidification process based on proton release into the rhizosphere, making nutrients more available for plants, was very well demonstrated under nutrient limiting conditions and showed a decrease in the soil pH. The ability of plants to acidify their medium is considered a good criterion of tolerance to nutrient deficiency stress especially in the case of iron (Fe). New insights into the role of the H⁺-ATPases in rhizosphere acidification using genetic tools are available. In the present review, we summarize the implication of rhizosphere acidification in nutrient uptake, with a special focus on potassium and phosphorus as important macroelements and iron, a key micronutrient involved in many plant physiological and biochemical processes.

2 Rhizosphere Acidification and Nutrient Uptake: Role of the Pump H⁺ATPases

According to Hinsinger (1998), plant nutrition is not only linked to plant physiology but includes all the processes that occur in the rhizosphere before the uptake of the required nutrient. Plants are able to change the pH of the rhizosphere during their growth and development. Such a property was well documented in the literature (Haynes 1983; Marschner et al. 1986; Nye 1981). When plants take up cations excessively from the soil solution, an increase of the proton extrusion is observed (Glass et al. 1981) leading to rhizosphere acidification. Indeed, the root hairs, the first organs that keep direct contact with the soil solution, are the site of noticeable proton flux detected by the use of microelectrode vibration (Palmgren 2001). According to Ruiz et al. (2002), the plasma membrane H⁺-ATPase has a crucial role in ion transport and a positive correlation between H⁺-ATPase activity and the concentrations of cation was found in roots (Ruiz et al. 2002). Such proton release is sustained by the activities of proton pumps located at the plasma membrane (Samuels et al. 1992; Jahn et al. 1998). It is well established that the major driving force for the cation and anion transport across the plasma membrane is the active extrusion of protons (H⁺) due to the activity of proton pump ATPases located at the root plasmalemma. In fact, ions are taken from the soil solution and transported into the root cells prior to their distribution in the different plant tissues. During their transport across the plant plasma membrane, ions are driven by an electrochemical gradient of protons as a result of the activity of plasma membrane H/Cation-ATPases (Miller and Aldrich 1996; Sussman 1994). The latter are qualified as powerhouses for nutrient uptake (Palmgren 2001).

Due to the importance of soil acidification in nutrient acquisition, among the promising solutions to improve nutrient uptake by plants is raising the pH of the rhizosphere making the major nutrients such as K, Ca, Mg, P, S, and N more available for plant roots as suggested by Dakora and Phillips (2002).

2.1 Potassium (K^+)

Potassium uptake by roots requires an exchange with an ion that has the same equivalent positive charge. Generally, one proton is exchanged against one ion of potassium. In fact, the uptake of potassium via channels depends on the electrochemical potentials across the plasma membrane (Serrano 1989). A positive correlation between potassium uptake and proton release was noted in barley by Glass et al. (1981), suggesting the importance of this process in potassium acquisition by plants. Bucker et al. (2006) reported a simultaneous extrusion of protons and K^+ influx from the solution in rice, revealing a linear relationship between H^+ pumping and K^+ uptake by this species. It has been suggested that plants acquire potassium from soil through an active process called symport which depends on the gradient of proton via the plasma membrane as revealed by Sze et al. (1999). The occurrence of the H^+/K^+ symporter in the plasma membrane contributes to the K^+ accumulation under potassium shortage conditions (Maathuis and Sanders 1994; Schachtman and Schroeder 1994), provides strong evidence for exchange of the K^+ ion over a proton, and leads to rhizosphere acidification.

The establishment of this proton gradient creates a proton motive force responsible for the transport of cations (Rodriguez-Navarro 2000) such as monovalent ones, like potassium. Such a process is due to the activities of plasma membrane H^+ -ATPases. The role of these proton pumps (H^+ -ATPases) in K^+ absorption was well demonstrated in a previous work by Minjian et al. (2007). It was also proved that K^+ uptake by plants was correlated with high activity of plasma membrane H^+ -ATPase (Briskin and Hanson 1992). According to these authors, the plasma membrane H^+ -ATPase might conduct an antiport transport H^+/K^+ that exchanges H^+ over K^+ , thus contributing to K^+ uptake. Moreover, the extracellular acidification based on H^+ pump activities was found to stimulate some transporters such as the symport K^+/H^+ . This finding was later confirmed by the identification of genes encoding some potassium transporters in many species including LeHAK5 in tomato (Wang et al. 2002), HvHAK1 in barley (Santa-Maria et al. 1997), CaHAK1 in pepper (Martínez-Cordero et al. 2005), and OsHAK1 in rice (Bañuelos et al. 2002). Furthermore, the activation of the H^+ -ATPase is necessary for K absorption by roots, as suggested by Minjian et al. (2007) who demonstrated that K^+ uptake by maize depends on the activity of proton pump H^+ -ATPase and on a specific K^+ transporter located at the membrane. In fact, the activity of such pumps generated an electrochemical gradient established by the liberation of protons H^+ , a determinant process in the K^+ acquisition by plants. These pumps were strongly stimulated when plants were subjected to limited K^+ supply and resulted in an acidification of the extracellular medium. In fact, the activity

of such pumps increased under K^+ deficiency conditions leading to an increase of cation exchange capacity. A decrease of the pH surrounding the roots and an influx of K^+ into the roots occurred simultaneously (Chen and Gabelman 2000). Several studies showed that following a few minutes of reduction of the external K^+ concentrations, a hyperpolarization of the root membrane potential was noted (Maathuis and Sanders 1993; Nieves-Cordones et al. 2008). This phenomenon is considered as the first response to potassium deficiency which was accompanied by an important release of protons into the external medium and to an acidification of the rhizosphere (Behl and Raschke 1987). These two physiological responses are generated by the activity of the H^+ -ATPase pump at the plasma membrane via pumping of H^+ from the cytoplasm to the apoplasm (Palmgren 2001). Such physiological responses are in favor of the activation of the K^+ channels and transporters resulting in an important influx of K^+ into the roots. Furthermore, the rate of proton excreted is considered as criteria to evaluate potassium deficiency tolerance in crops. Thus, one of the criteria of tolerance to K^+ deficiency is the capacity of the plant to expulse H^+ . K^+ uptake efficiency in tomato was correlated with a high K^+ influx that was associated with low pH value (Chen and Gabelman 2000). Several proton pumps were identified in different plant species (Serrano 1989; Nardi et al. 2002). The role of these H^+ -ATPases was studied well by the application of different pump inhibitors or stimulators such as vanadate and fusicoccin. The vendetta is known to block the activity of H^+ -ATPases. For example, in rice, the addition of vanadate in the nutrient solution totally blocked the activity of the H^+ -ATPases, and led to an inhibition of potassium uptake by the plants (Bucker et al. 2006).

2.2 *Phosphorus (P)*

The involvement of rhizosphere acidification on P mobilization was described in bacteria, fungi, and plants. The bacteria PSB or the fungi PSF can mobilize soil P by the release of some compounds and via the acidification of the soil (Jones and Oburger 2011). According to the literature, the excretion of H^+ into the rhizosphere improves phosphorus (P) availability in the soil (Neumann and Römheld 1999; Hinsinger et al. 2003, 2011). In fact, rhizosphere acidification led to both enhancement of P mobilization from soil and makes it available for plants (Hinsinger 2001; Hinsinger et al. 2011; Hinsinger et al. 2009; Zhang et al. 2010).

As for potassium, the uptake of P involves the H^+/Pi symport system as suggested by Ai et al. (2009) and is coupled to H^+ transport (Sakano 1990). Thus, under P deficiency conditions, this process increased notably. In rice, the mobilization of P is due to rhizosphere acidification via the liberation of H^+ from the roots. Such behavior occurred to maintain equilibrium between excessive uptake of cations against anions (Saleque and Kirk 1995). P starvation induced root H^+ release (Li et al. 2004) leading to rhizosphere acidification. This was very well documented (Neumann and Römheld 1999; Raghothama 1999; Hinsinger 2001; Richardson et al. 2001; Vance et al. 2003; Tang et al. 2004; Raghothama and Karthikeyan 2005).

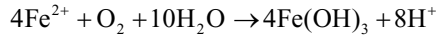
In the case of white lupin and *Lupinus albus*, an acidification of the external medium occurred under P starvation (Racette et al. 1990). Shen et al. (2006) demonstrated that in soybean and *Arabidopsis* roots conducted under P deficiency conditions, an increase of the plasma membrane H⁺-ATPase (PM H⁺-ATPase) activity was noted. In rice, the increase of the PM H⁺-ATPase contributed to the rhizosphere acidification (Shen et al. 2006; Zhang et al. 2011) and sustained the transport of this nutrient via the plasma membrane.

The ability of plants to acidify the rhizosphere is considered criteria to evaluate the P tolerance degree of plants inasmuch as species differ in their capacity to enhance PM H⁺-ATPase under P limiting availability (Shen et al. 2006). P is present in the soil solution at low concentrations (μM). Thus, systems with high affinity are involved in its transport. Furthermore, Pi uptake occurred against a chemical potential gradient across the root plasma membrane (Shen et al. 2011) and it is mediated by a symporter Pi/H⁺. Recently, this latter was characterized as a member of the PHT1 gene family because of the positive correlation found between PHT1 alteration and the decrease of P uptake (Ai et al. 2009).

According to Jing et al. (2010), the management of the rhizosphere through the optimization of N forms and the P input could be useful to stimulate both root proliferation and the acidification process. Calcareous soils are characterized by high pH. Thus, plants grown in such kind of soils suffered often from low nutrient availability. There, phosphorus can be present as hydroxyapatite (HAP), a stable form of dicalcium phosphate (DCP), which can be dissolved rapidly as the soil pH decreases (Wang and Nancollas 2008). Such a finding suggests the importance of the rhizosphere acidification process as an efficient strategy for mobilizing soil P from calcareous soils (Shen et al. 2011). Consequently, to improve the productivity of such areas, an application of ammonium could be effective inasmuch as NH₄⁺ induces an acidification of the rhizosphere doing so, the nutrients ready for root uptake especially in the case of phosphorus. It was found that the positive effect of localized NH₄⁺ and P on plant growth is related to its role in lowering the pH rhizosphere due to the presence of ammonium and in increasing the acquisition of P by roots (Bloom et al. 2003; Miller and Cramer 2004; Jing et al. 2010). In maize, application of ammonium decreased the rhizosphere pH by 3 pH units indicating that the localized application of P combined with ammonium improves nutrient uptake due to the stimulation of the rhizosphere acidification (Jing et al. 2010).

The coculture of cereals and legumes assumed that this association was beneficial for a cereal P status because of the high aptitude of legume species to release a larger amount of protons as revealed by Tang et al. (1997) and Hinsinger et al. (2003). In a P-deficient intercropping system, P can be mobilized by legumes because these plants are able to acidify the rhizosphere due to their capacity to release protons and this occurred during P deficiency and N fixation (Li et al. 2007; Zhang et al. 2010). Because cereals are not very efficient in lowering the pH of the rhizosphere, an intercropped cereal/legume resulted in an intermediate pH (Cu et al. 2005; Li et al. 2008a, b). In fact, the form of N, ammonium (NH₄⁺), nitrate (NO₃⁻), or dinitrogen (N₂), and its uptake are known to induce several changes in the pH of the rhizosphere and therefore affect P availability for plants as shown by Hinsinger et al. (2003).

In flooded soils, among the successful processes used by plants to mobilize phosphorus, we cited the acidification of the rhizosphere (Saleque and Kirk 1995) which involved the oxygen released from roots that oxidizes Fe^{2+} to release two protons according to the reaction:



The inequality of the uptake of cation over anion, especially in the presence of NH_4^+ ions in reduced soil, was shown by Begg et al. (1994). In this case, an important release of protons into the rhizosphere was noted.

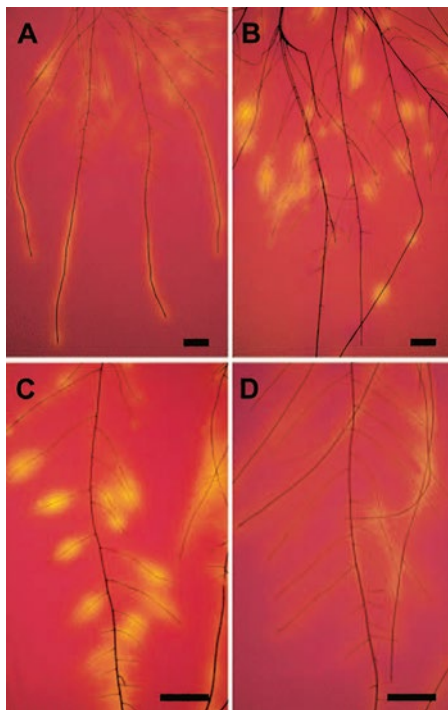
2.3 Iron (Fe)

The acidification process is among the important mechanisms involved in Fe uptake by plants and characterized the Strategy I plants, especially, under the iron (Fe) deficiency. According to Liang et al. (2013), Fe acquisition in plants adopting Strategy I, is achieved by the combined functions of two components: the ferric chelate reductase and the proton-extruding H^+ -ATPase. In fact, this class of plants includes dicotyledonous and nongraminous monocotyledons known by their ability to induce an active proton extrusion via the increase of H^+ -ATPase activity in root plasmalemma leading to an acidification of the rhizosphere (Zocchi and Cocucci 1990). The decrease of the rhizosphere pH is a typical root response of dicotyledonous species under Fe deficiency conditions (Zocchi and Cocucci 1990; Donnini et al. 2009). Such a physiological response is adopted by many plant species. Peanut increases the acidification of the rhizosphere via the release of protons from the roots (Zuo et al. 2000, 2003). Lowering the rhizosphere pH is considered the most important component conferring a good adaptive response to Fe-deficiency in Strategy I plants (Santi et al. 2005). As reported by Schmidt et al. (2003), the density of the plasma membrane ATPase (PM H^+ -ATPase) was twofold higher in Fe-deficient roots of tomato leading to a very low pH of the rhizosphere.

As for K^+ and phosphorus, the ability of plants to lower the pH of the nutrient solution is used as an important criterion to screen tolerant genotypes to Fe deficiency (Dell'Orto et al. 2000). Such findings are very well documented in many species such as peach (Molassiotis et al. 2006), kiwifruit (Rombolà et al. 2002), pea (Jellali et al. 2010), and medicago (M'sehli et al. 2011), and was observed for both glycophyte and halophyte species. A recent comprehensive study in a perennial halophyte *Suaeda fruticosa* revealed that this species has a great ability to acidify the external medium (Houmani et al. 2012, 2015).

The release of protons into the rhizosphere occurred via the activation of a plasma membrane proton pump (H^+ -ATPase) which is stimulated under such conditions (Schmidt 2003; Dell'Orto et al. 2000). In poor Fe soils, and in order to increase Fe availability, Strategy I plants activate a series of plasma membrane proton pumps (H^+ -ATPases) (Zocchi 2006; Kim and Guerinot 2007) resulting in the establishment

Fig. 2 Visualization of root medium acidification in Fe-sufficient (a), Fe-deficient (b) plants, and in roots using split-root system (plants grown in iron-containing medium) (c), or in Fe-free nutrient solution (d) using Bromocresol Purple as a pH indicator. The yellow color corresponds to high proton release (Schmidt et al. 2003)



of an electrochemical gradient (Palmgren 2001), and leading to an increase of ferric Fe solubility (Walker and Connolly 2008). Using immunolabeling methods, it was possible to detect the site of the H^+ -ATPase enzyme at the deficient subapical root zones (Dell'Orto et al. 2002; Schmidt et al. 2003; Fig. 2). The activity of these pumps has been controlled genetically due to the transcriptional upregulation of a family of HA genes, which were identified by Dell'Orto et al. (2002) in Fe-deficient cucumber plants.

The H^+ -ATPase activity is the key component of Strategy I plant responses to Fe shortage conditions. In fact, the differences in plant responses to Fe deficiency are particularly attributed to H^+ extrusion (Schmidt 1999) rather than to FC-R activity. Recently, Slatni et al. (2011) showed an increase of the H^+ -ATPase activity under Fe starvation in nodules of common bean plants. The same authors have demonstrated that this H^+ -ATPase protein was accumulated in Fe-deficient nodules of the *Flamingo common bean* variety and participated in the uptake of Fe by the nodules from the soil solution. It has been shown that high H^+ extrusion activity was positively correlated with a strong induction of PEPC activity in many plant species including *Phaseolus vulgaris* (Bienfait et al. 1989), *Capsicum annuum* (Landsberg 1986), and *Beta vulgaris* (López-Millán et al. 2000); by contrast, in plants with a low H^+ -ATPase activity, PEPC activity was not induced under the same conditions (Zocchi et al. 2007; M'sehli et al. 2009).

3 New Advances in the Identification of H⁺ATPases Using Molecular Tools

Plasma membrane H/Cation-ATPases are known as proton pumps localized in the plasma membrane of plants and are driven by the hydrolysis of ATP as a principal source of energy. H/C-ATPases play an important role in nutrient acquisition and translocation into the cell because they represent the major source of energy necessary for nutrient uptake and transport through the roots. This energy is generated by the extrusion of a positive charge across the plasma membrane. When protons are excreted into the external medium, an electrochemical gradient is established on either side of the membrane and cations can enter into the cell through the attraction due to the differences of charge and then are transported via different proteins.

Because of the recent progress in biotechnology, it is possible to provide new insights into the role of those H⁺/Cation-ATPases using the plant model *Arabidopsis thaliana* and the new advances in genetic manipulation. In fact, by using reverse genetic methods, a complete H/Cation-ATPase gene family was identified in *Arabidopsis* giving us good information regarding genetic control of the activity of these pumps at the posttranslational level (Palmgren 2001). Several candidate H/Cation-ATPases localized at the root epidermal cells and root hairs were identified and were considered as the principal drivers for nutrient uptake from the soil to the roots (Palmgren 2001). Using the immunoblot technique, it was possible to detect high amounts of H/Cation-ATPase apart from the epidermal cells of roots (Parets-Soler et al. 1990; Jahn et al. 1998; Figs. 3 and 4). Such a localization suggests their role in the active loading of solutes into the xylem sap (Parets-Soler et al. 1990). As described by Oufattole et al. (2000), plant plasma membrane H⁺-ATPases (PM H⁺-ATPases) are encoded by a multigene family. Recently, 12 HC-ATPase genes were cloned in *Arabidopsis* (Palmgren 2001). In fact, it was shown that the activity of H⁺-ATPase is upregulated by an H⁺-ATPase AtAHA gene as pointed out by several biologists (Colangelo and Guerinot 2004; Walker and Connolly 2008; Buckhout et al. 2009; García et al. 2011). Recently, an H⁺-ATPase (AHA) protein family was identified in *Arabidopsis* and was shown to be responsible for soil acidification (Ivanov et al. 2012). These data were confirmed using a mutant of AHA2 which lost its activity (Sussman 1994; Santi and Schmidt 2009). These authors demonstrated that the H⁺-ATPase AHA₂ is responsible for the main acidification activity under iron deficiency conditions and the gene responsible for AHA₂ activity is regulated under these same conditions. A family of genes encoding H⁺-ATPase proteins was also identified in cucumber H⁺-ATPase [CsHA1; Santi et al. 2005; Santi and Schmidt 2008] and (CsHA₂ and CsHA₃; Młodzińska et al. 2010), and are responsible for the rhizosphere acidification process. Using a semi-quantitative reverse transcriptase (RT)-PCR and quantitative real-time RT-PCR techniques, Santi et al. (2005) successfully identified two PM H⁺-ATPase cDNAs (CsHA₁ and CsHA₂) from Fe-deficient cucumber and found a high accumulation of CsHA1 gene transcripts in roots suggesting the genetic control of the pump proton activity under lowering Fe conditions. A new proteomic study identified a root V-ATPase implicated in the

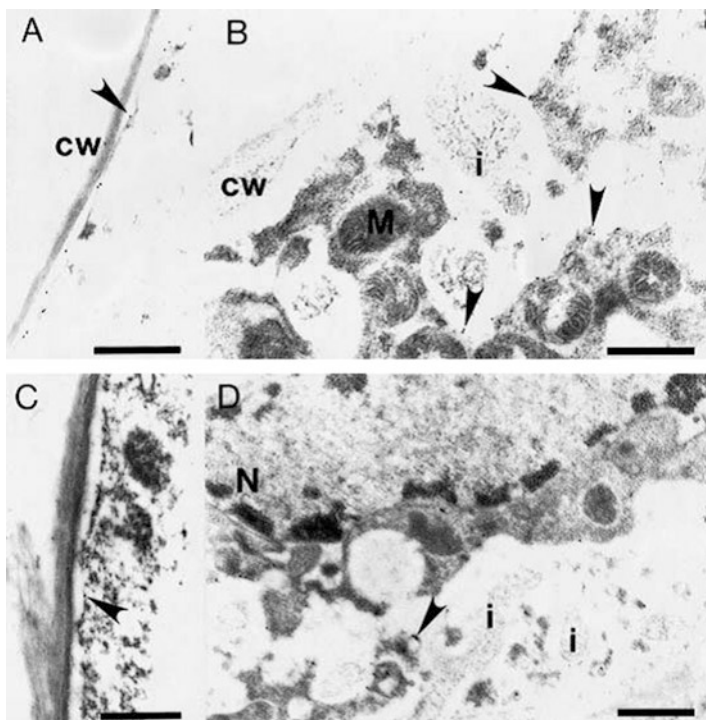


Fig. 3 Immunocytochemical localization of plasma membrane H^+ -ATPase in epidermal cells of tomato roots. (a) Rhizodermal cell of Fe-sufficient tomato plant. (b) Wall ingrowths of Fe-deficient root transfer cell. (c) Control root section treated only with a secondary antibody: a goat antimouse IgG, dilution 1:50. (d) Secondary wall ingrowths induced by exogenous application of 2,4-D. CW: cell wall; M: mitochondria; N: nucleus; I: invagination. (Schmidt et al. 2003)

responses of plants to Fe deficiency (Wang and Wu 2010; Lan et al. 2011). This protein was found to provide the necessary acidification for the induction of some physiological responses under Fe limitation conditions especially cell elongation and new root development.

4 Effect of Salinity on Rhizosphere Acidification

Salinity is a major constraint affecting plant growth and productivity (Hakeem et al. 2012, 2013). In general, saline soils are generally characterized by the predominance of salt toxic ions (mainly Na^+ and Cl^-) and by their low nutrient availability (Inal and Gunes 2008). This situation is probably attributed to the effect of salt ions on the H^+ -ATPases and resulting in an inhibition of rhizosphere acidification. Rhizosphere acidification is a central mechanism by which plants can take mineral nutrients from the soil solution. Nevertheless, the activity of these pumps is

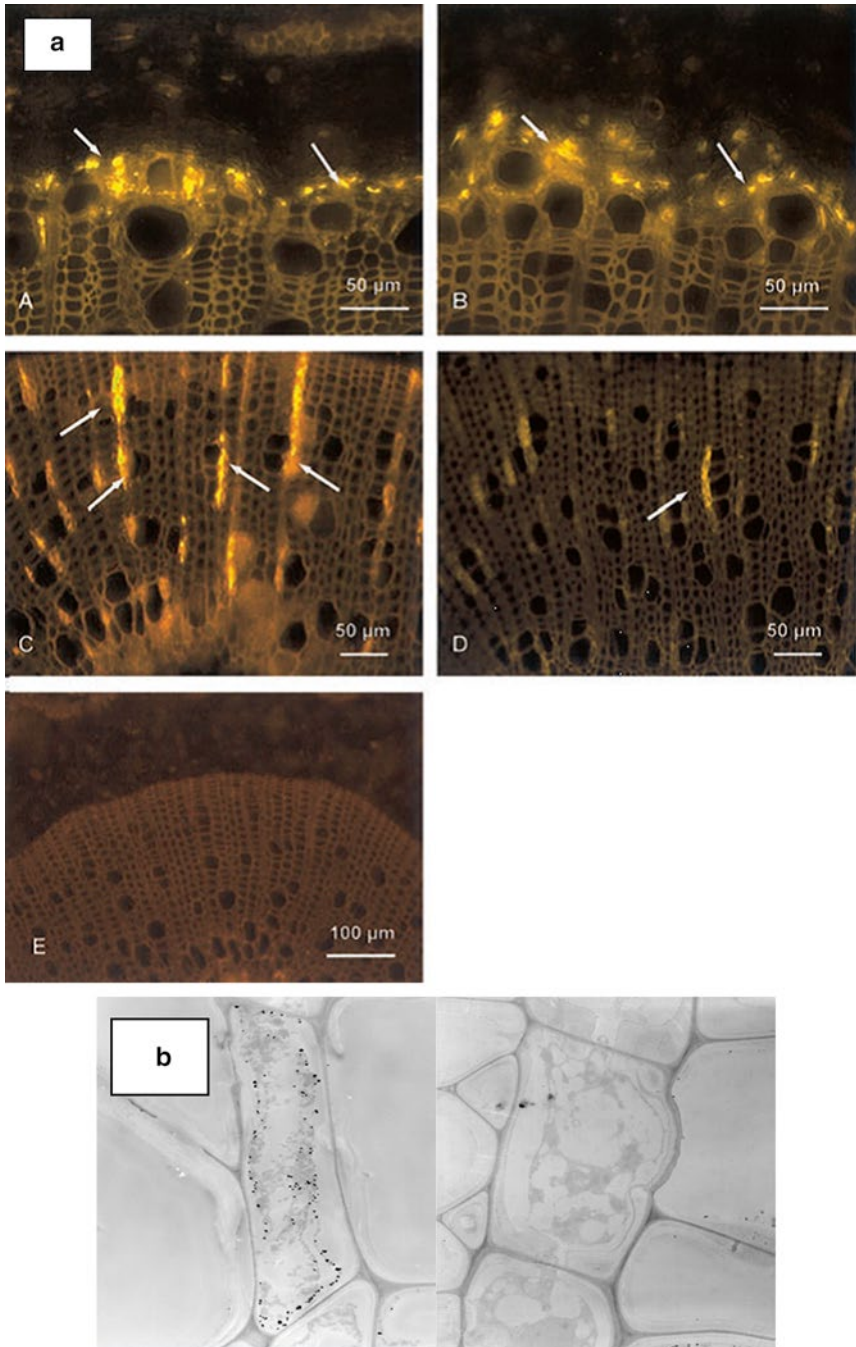


Fig. 4 (A) Localization of PM H⁺-ATPases in poplar stems using the monoclonal antibody 46 E5 B11 in plants. Similar labeling with low (a) or high K⁺ supply (b). (B) Cellular localization of PM H⁺-ATPases using the monoclonal antibody 46 E5 B11. (Arend et al. 2004)

dependent on the soil structure and properties. In fact, salinity can negatively affect the H⁺ATPase function and lead to an inhibition of the proton release and consequently inhibit nutrient uptake by plants. It was demonstrated that a moderate salt stress inhibited the rhizosphere acidification of many glycophyte species. For *Medicago ciliaris*, the application of 75 mM NaCl reduced the activity of PM H⁺-ATPase, resulting in a reduction of Fe uptake by this species (Rabhi et al. 2007; M'sehli et al. 2011). The study on some nutrient deficiency in halophytes, plants adapted to extreme environmental conditions, revealed that such kind of vegetation is able to maintain the uptake of nutrients from soil with high salinity levels. This behavior was proved under nutrient deficiency. A recent study (Houmani et al. 2015) of the halophyte *Suaeda fruticosa* under the combined effect of salt stress and iron deficiency showed that this species was able to maintain its acidification capacity under high salinity levels to maintain the uptake of this element of the external medium.

5 Conclusion

Rhizosphere acidification is an important natural process for nutrient uptake by plants. The liberation of the proton is in favor of the uptake of one cation. The proton release is essential for driving the transport of nutrients into the roots. This phenomenon is complex and is due to very consistent powerhouses, the H⁺-ATPases that function to assure an adequate nutrient supply for plants. The use of the new advances in genetic research gives new insights in the role of such proton pumps in mineral uptake and translocation from the rhizosphere into the roots. The role of H⁺-ATPases in higher plant nutrition was well demonstrated under limiting conditions of deficiency in several essential elements such as potassium, phosphorus, and iron.

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