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Nice to meet you: genetic, epigenetic and metabolic controls of plant perception of beneficial associative and endophytic diazotrophic bacteria in non-leguminous plants

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Abstract A wide range of rhizosphere diazotrophic bacteria are able to establish beneficial associations with plants, being able to associate to root surfaces or even endophytically colonize plant tissues. In common, both associative and endophytic types of colonization can result in beneficial outcomes to the plant leading to plant growth promotion, as well as increase in tolerance against biotic and abiotic stresses. An intriguing question in such associations is how plant cell surface perceives signals from other living organisms, thus sorting pathogens from beneficial ones, to transduce this information and activate proper responses that will finally culminate in plant adaptations to optimize their growth rates. This review focuses on the recent advances in the understanding of genetic and epigenetic controls of plant-bacteria signaling and recognition during beneficial associations with associative and endophytic diazotrophic bacteria. Finally, we propose that "soil–rhizosphere–rhizoplane–endophytes–plant" could be considered as a single coordinated unit with dynamic components that integrate the plant with the environment to generate adaptive responses in plants to improve growth. The homeostasis of the whole system should recruit different levels of regulation, and recognition between the parties in a given environment might be one of the crucial factors coordinating these adaptive plant responses.

Keywords Associative diazotrophic bacteria · Endophytic diazotrophic bacteria · Plant recognition · Plant receptors · Small RNAs

Introduction

Plants are constantly challenged by fluctuations in their environment and exposure to microorganisms in the rhizosphere. A wide range of rhizosphere microorganisms are able to establish beneficial associations with plants, being able to colonize root surfaces or even switch to endophytic lifestyles (Saharan and Nehra 2011). Some beneficial associations of plants with bacteria and fungi have been extensively characterized. The best-studied models of interaction are those with arbuscular mycorrhizal fungi (AMF) and with the rhizobial endosymbiont bacteria (REB) (Oldroyd 2013).

During plant interaction with AMF, plant allows fungal colonization into the inner root cortex, where arbuscules develop and mediate nutrient delivery to the plant (Schmitz and Harrison 2014). In the association between legumes and REB, bacteria have the ability to supply nitrogen (N) to plants through biological nitrogen fixation (BNF), being called diazotrophic (Maróti and Kondorosi 2014). When legumes interact with rhizobia, nodules are formed and provide a proper environment for bacterial nitrogen fixation since it restricts free flow of oxygen, an inhibitor of BNF enzymatic process (Dixon and Kahn 2004).

Other systems of N-fixing associations with non legumes have been described (Reinhold-Hurek and Hurek 2011), however they differ from rhizobia, as bacteria do not reside intracellularly in living plant cells and their colonization does not induce the formation of any visible differentiated plant structure (Boddey et al. 1995;

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Reinhold-Hurek and Hurek 1998; James and Olivares 1998; Baldani and Baldani 2005; Monteiro et al. 2012). Several data demonstrate significant rates of BNF related with associative and endophytic diazotrophic bacteria, here named AEDB, leading to a reduction in the use of N fertilization and increase in plant yield (Döbereiner 1997; Dobbelaere et al. 2003; Vessey 2003; Bhattacharyya and Jha 2012; Carvalho et al. 2014). As natural N supply is a limiting factor in plant yield, the association of non legumes with AEDB may represent a promising alternative to the environmental and economical costs of the use of chemical N fertilizers (Robertson and Vitousek 2009).

An intriguing question in such rhizospheric associations is how plant senses signals from other living organisms, thus sorting pathogens from beneficial ones, to transduce this information and activate proper responses that will finally culminate in plant adaptations to optimize their growth rates. As observed for AMF and REB associations, there must be a chemical communication between the microorganism in the rhizosphere and the host plant root (Oldroyd 2013). Plant root signals are released for microorganism attraction, and in turn, plant receptors recognize microorganisms' factors activating symbiosis-signaling pathways (Oldroyd 2013). This review focuses on the recent advances in the understanding of genetic, epigenetic and metabolic controls of plant-bacteria signaling and recognition during AEDB associations.

Features of bacterial associative and endophytic diazotrophic associations

Different from nodulating rhizobial associations, a wide range of groups of diazotrophic bacteria may have the ability to establish associative and endophytic associations with plants, including alpha-, beta- and gamma-Proteobacteria (Boddey et al. 1995; Baldani and Baldani 2005). Among the best described genera are species of *Azospirillum, Azorhizobium, Azoarcus, Burkholderia, Citrobacter, Enterobacter, Gluconacetobacter, Herbaspirillum, Klebsiella and Pseudomonas* (Vessey 2003; Kennedy et al. 2004; Magnani et al. 2010; Santi et al. 2013). Interestingly, different species of rhizobia and bradyrhizobia have been found associated with non-leguminous plants such as sugarcane (Beneduzi et al. 2013; Rouws et al. 2014).

Considering the niche of colonization, these bacteria can be classified as associative when they colonize the rhizoplane (root's surface), especially of root hair, elongation zones and regions of cracks formed during lateral root formation (James 2000; Rosenblueth and Martínez-Romero 2006; Monteiro et al. 2012). The endophytic bacteria explore tissues within the root, as root cortex and stele, living in intercellular spaces and within xylem vessels (James 2000; Rosenblueth and Martínez-Romero 2006; Carvalho et al. 2011; Reinhold-Hurek and Hurek 2011). In common, both associative and endophytic types of colonization can result in beneficial outcomes to the plant leading to plant growth promotion as significant increases in the plant's height and biomass, root length, dry-matter production and grain yield are observed, as well as increase in tolerance against biotic and abiotic stresses (Dobbelaere et al. 2001; Creus et al. 2004; Arencibia et al. 2006; Rosenblueth and Martínez-Romero 2006; Bally and Elmerich 2007; Spaepen et al. 2008; Richardson et al. 2009; Saha et al. 2013; Camilios-Neto et al. 2014; Vargas et al. 2014).

However, the assignment as associative or endophytic colonization is not always well defined since bacterial niches and numbers can be dynamically controlled during plant-bacteria interaction, in response to plant and environmental signals (Urquiaga et al. 1992; Schloter and Hartmann 1998; Oliveira et al. 2003; Vargas et al. 2014; Carvalho et al. 2014). In wheat cultivars inoculated with *Azospirillum brasilense* strains Sp7, Sp245 and Wa5, all the bacteria strains could associate with roots in significant high numbers, however only Sp245 strain was also capable of endophytically colonize the plant (Schloter and Hartmann 1998). Furthermore, some AEDB not always establish a beneficial association with plants, as *Herbaspirillum rubrisubalbicans* interaction with some sugarcane cultivars resulted in mottled stripe disease typical symptoms (Olivares et al. 1997). BNF rates in sugarcane plants growing in soils with different N levels were more efficient in soils with low nitrogen content than in N rich soils (Oliveira et al. 2003). In addition, sugarcane roots inoculated with *Gluconacetobacter diazotrophicus* and submitted to water deficit showed higher levels of colonization than inoculated roots growing in normal watering conditions (Vargas et al. 2014). Hence, the efficiency and possibly the type of beneficial output provided during plant-AEDB association might also be controlled by the environment and physiology of the plant-bacteria partners (Oliveira et al. 2003; Carvalho et al. 2011; Vargas et al. 2014; Carvalho et al. 2014).

Therefore, we can speculate that "soil–rhizosphere–rhizoplane–endophytes–plant" could be considered as a single coordinated unit with dynamic components that integrate the plant with the environment to generate adaptive responses in plants to improve grow (Fig. 1). The homeostasis of the whole systems should recruit different levels of regulation. Bacterial colonization is controlled by plant and soil conditions, and associated bacteria might influence plant responses to soil conditions to improve plant growth, by providing nutrients and by increasing tolerance to stresses, in a dynamic way that would be adjusted during plant life cycle depending on the plant

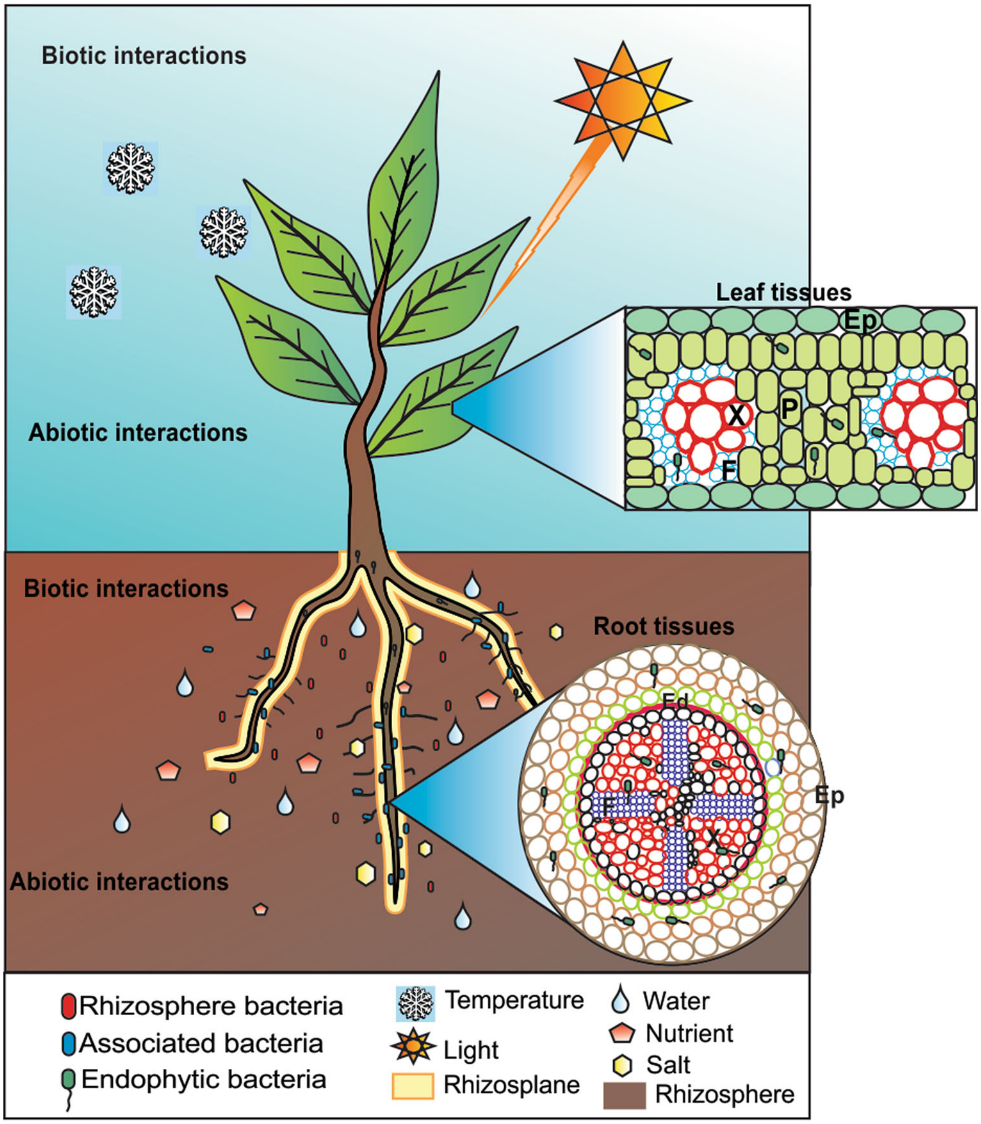

Fig. 1 Overview of the interaction between plants and beneficial bacteria. The interaction of beneficial bacteria with plants can occur in three main forms: bacteria are present in the rhizosphere, or they are associated to root surface, or they colonize root and shoot tissues. The specific bacterial niches are controlled by plant and soil conditions, and associated bacteria might influence plant responses to soil conditions to improve plant growth. In this context, the "soil–rhizosphere–rhizoplane–endophytes–plant" might function as a single coordinated unit with dynamic components that integrate the plant with the environment to generate adaptive responses in plants to improve growth. *Ed* endodermis; *Ep* epidermis; *F* floem; *P* medular parenchyma; *X* xylem

physiology and needs. Consequently, recognition between the parties in a given environment might be one of the crucial factors coordinating these adaptive plant responses.

Plant-bacteria recognition

The first steps of plant colonization by AEDB have been well studied (Reinhold-Hurek and Hurek 1998; James and Olivares 1998; James et al. 2001; Rosenblueth and Martínez-Romero 2006; Compant et al. 2010). First, plant attracts bacteria by the release of root exudates. After migration towards plant root, bacteria adhere to the surface of roots through exopolysaccharides (EPS) and lipopolysaccharides (LPS) present in bacteria wall (Rosenblueth and Martínez-Romero 2006; Reinhold-Hurek and Hurek 2011). At this point, several mechanisms must be regulated in order to provide an appropriate recognition process, distinguishing beneficial and pathogenic interactions. This process might depend on signals released by bacteria as well as in mechanisms of plant recognition of these signals, such as plant receptors.

An important question is how and when plants perceive the diazotrophic bacteria as beneficial. Both pathogenic and beneficial bacteria are initially recognized as potential harmful invaders, allowing the control of bacterial colonization. Some works using genomic approaches have demonstrated that plant signaling responses activated by pathogenic and beneficial interactions share some overlap (Reymond et al. 2004; Verhagen et al. 2004; De Vos et al. 2005; Sanchez et al. 2005; Kempema et al. 2007), suggesting that adaptive response of the plant must be fine-tuned to balance between protection against pathogens and acquisition of benefits from beneficial bacteria.

Symbionts signals

Plants, as well as observed in animals, present an innate immune system responsible for recognizing invading organisms (Pel and Pieterse 2013). This process involves the perception of non-self molecules known as microbe- or pathogens-associated molecular patterns (MAMPs or PAMPs) (Gómez-Gómez and Boller 2002; Jones and Dangl 2006). Some of these MAMPs have already been identified although most of the knowledge came from pathogenic interactions (Box 1) (Pel and Pieterse 2013).

Flagellin is the main structural protein of bacterial flagella, and is one of the best-studied bacterial proteins recognized as a MAMP (Boller and Felix 2009) for both beneficial and pathogenic bacteria. Purified flagellin elicits an oxidative burst, callose deposition and synthesis of antimicrobial proteins in plant cells (Felix et al. 1999; Gómez-Gómez and Boller 2000). Flg22, a synthetic 22-amino-acid peptide that corresponds to flagellin immunogenic N-terminus, is a potent elicitor of defense responses in *Arabidopsis* and other plant species (Felix et al. 1999).

The various AEDB may present single polar flagella, primarily used for swimming, and/or multiple lateral flagella, that allow the bacterium to swarm over a solid surface. An *A. brasilense* mutant lacking both polar and/or lateral flagella was completely non-motile and also deficient in adhesion to wheat root surface (Croes et al. 1993), suggesting that this structure might be important for bacterial association to the root surface. In contrast, the *Azoarcus* sp. mutant in flagellin was still able to establish microcolonies on rice root surface but showed significantly reduced root endophytic colonization, and did not activate defense-related responses, suggesting that flagellin is mainly required for endophytic colonization in the *Azoarcus*-rice interaction (Buschart et al. 2012). These studies indicate that the flagella could be important in AEDB associations for mobility at rhizosphere and eventually inside plant tissues, but it is still unclear whether flagellin would play a major role as a MAMP. In rhizobia association studies, the general elicitor flg22 activated defense responses in *Lotus japonicus* roots, which inhibited infection by the nodulating diazotrophic rhizobia and delayed nodule organogenesis, suggesting a negative role of flagellin in the initial rhizobium–legume interaction (Lopez-Gomez et al. 2012). However, defense and symbiotic pathways overlapped, and the latter was dominant allowing symbiosis to be established further, which was consistently followed by down-regulation of the mRNA levels of the flg22 receptor FLS2 (Lopez-Gomez et al. 2012). Similiar mechanisms could also be operating during AEDB associations. However, another unresolved questions are whether all different species of AEDB have flagella at all stages of plant colonization, and whether their mechanisms of signaling are regulating the initial colonization of roots and/or the endophytic multiplication.

Box 1 Pathogen recognition in plants

Plant can naturally interact with a wide range of microorganisms that can be harmful or beneficial. In order to distinguish pathogens and beneficial microorganisms, plants evolved an efficient system of recognition. Although little is known about the perception of beneficial microorganisms, interaction with pathogens has been vastly investigated. The first steps of response consist of the recognition of pathogens-associated molecular patterns (PAMPs). Different molecules represent PAMPs like flagellin, peptidoglycans (PGNs) and lipopolysaccharides (LPSs). The activation of this immune system occurs through recognition of PAMPs by receptors localized in the plant plasma membrane (pattern recognition receptors—PRRs). Several proteins families constitute PRRs, among them receptor-like kinases (RLKs) and receptor-like proteins (RLPs). The extracellular domains of PRRs are involved in MAMP recognition and confer ligand specificities. While lysine motifs (LysM) or lectin motifs are common ectodomains in RLKs and RLPs, the leucine-rich repeat (LRR) extracellular motif are the most represented in plants. PRR-mediated microbe sensing induces a broad variety of defense responses commonly referred to as PAMP-triggered immunity (PTI). PTI is a defense program with complex early signaling events leading to the massive transcriptional reprogramming that initiates defense responses such as stomatal closure, cell wall strengthening, and production of antimicrobial compounds. However, successful pathogens suppress or interfere with the PTI responses by secreting different compounds such as effectors, proteases or toxins, facilitating host colonization. In this context, plants evolved specific intracellular receptor (R) proteins that detect these effectors, which initiate a defense program in the so-called effector-triggered immunity (ETI). These R proteins are generally cytosolic receptors like nucleotide binding site (NBS) receptors, and this recognition also triggers different responses in order to combat the pathogens.

One of the first crucial events in the plant-AEDB associations is the bacteria attachment to the host root. Type IV pili (TFP) is essential for bacterial adherence and colonization of host cell surfaces, as well as in twitching motility (Böhm et al. 2014). In AEDB, TFP role has been studied in *Azoarcus* sp. mutant in pilin, a major component of TFP, which showed significantly reduced adhesion and colonization of rice roots, suggesting the importance of TFP in the first steps of this interaction (Dörr et al. 1998).

Another bacterial factor commonly recognized by plants is LPS, and its mechanisms of action have mostly been characterized during interaction with plant pathogens. LPS are glucoconjugates present in the outer membrane of Gram-negative bacteria that contribute to the structure of the bacterial envelope and offer protection against antimicrobial compounds (Pel and Pieterse 2013). In beneficial associations, LPSs have been related to induction of resistance against pathogens and also with endophytic and epiphytic colonization (de Weger et al. 1989; Duijff et al. 1997). LPS is involved in colonization of the tomato roots by *Pseudomonas fluorescens* WCS417r, as the bacteria with a mutational variant of LPS colonizes tomato root in lower numbers than wild type bacteria (Duijff et al. 1997). These mutational variant also was unable to activate plant defense responses suggesting that recognition of the bacteria by the plant was compromised.

In AEDB associations, LPS is also required for bacterial colonization process. *Herbaspirillum seropedicae* mutant strains impaired in LPS biosynthesis showed a severe reduction in attachment to the maize root surface compared with the wild-type strain (Balsanelli et al. 2010). Other *H. seropedicae* mutants, with altered LPS profile, also showed a reduction in the capacity to endophytically colonize maize (Tadra-Sfeir et al. 2011). The importance of LPS in these first steps of plant-bacteria recognition is reinforced by the fact that several genes involved in LPS biosynthesis are transcriptionally regulated by plant-derived signals, such as flavonoids, known to be involved in chemotaxis (Balsanelli et al. 2010; Tadra-Sfeir et al. 2011). The data indicate that during plant-AEDB recognition process, plant produces some compounds that regulate bacterial metabolism, including LPS. These components will be important for bacterial recognition and, consequently, for the following plant colonization.

EPSs represent another group of signals involved in the first steps of colonization, and they also participate in biofilm formation (Rodríguez-Navarro et al. 2007; Meneses et al. 2011). In rhizobia associations, EPS is important for several processes including bacterial host entrance (Rodríguez-Navarro et al. 2007). EPS production has been demonstrated for several AEDB genera such as *Azoarcus*, *Azospirillum*, *Burkholderia* and *Gluconacetobacter* (Hurek and Reinhold-Hurek 2003; Valverde et al. 2008; Hallack et al. 2009). A *G. diazotrophicus* mutant, defective in EPS production, was incapable to form biofilm and it was also affected in attachment to rice root surface and in endophytic colonization (Meneses et al. 2011). This data suggests that EPS biosynthesis is required for biofilm formation and plant colonization during early stages of plant recognition of a beneficial diazotrophic bacterium.

The type III protein secretion system (TTSS) is used by bacteria to deliver effector proteins into cytoplasm of host cells, playing an important role in plant-microorganism recognition (Büttner and Bonas 2002; Greenberg and Vinatzer 2003). Genomic analyses identified genes homologous to the TTSS in some AEDB like *A. brasilense* and *H. seropedicae*, raising the possibility that it may be involved in their interaction with host plants (Steenhoudt and Vanderleyden 2000; Monteiro et al. 2012). *H. rubrisubalbicans* mutants in the TTSS showed reduced capacity to colonize rice and maize plants, suggesting that TTSS is involved in the endophytic colonization (Schmidt et al. 2012). In the genus *Pseudomonas,* that includes some known AEBD, the regulatory, structural and effector genes of TTSS are closely related to those of pathogenic bacteria (Preston et al. 2001; Wolfgang et al. 2003). Therefore, it seems likely that TTSSs from beneficial bacteria promote colonization of host plants in a similar way as those from plant pathogens. The translocated effectors might change the plant cellular metabolism to allow colonization, for the benefit of the bacterium in the case of pathogens, or for both partners, in the case of symbiotic and associative bacteria (Hacker and Carniel 2001; Grant et al. 2006). However, several AEDB do not harbor TTSS genes in their genomes, indicating that the various bacteria might use distinct signaling pathways to establish an endophytic type of association with plants.

Many beneficial bacteria, including AEDB, use acylated homoserine lactones (AHLs) to monitor the external environment and the proximity of other bacteria (Loh et al. 2002; Von Bodman et al. 2003). AHLs are involved in the quorum sensing (QS), a signaling mechanism that control the expression of several genes important for microbial interactions, host colonization and stress survival (Hense et al. 2007; Atkinson and Williams 2009). AHLs from the growth promoting bacteria *Serratia liquefaciens* MG11 and *Pseudomonas putida* caused specific systemic responses, reducing cell death after infection with fungal pathogen *Alternaria alternata* (Schuhegger et al. 2006). *G. diazotrophicus* produces eight different AHLs that act as signals for QS (Nieto-Peñalver et al. 2012; Bertini et al. 2014). Mathesius et al. (2003) used proteomics to show that the model legume *Medicago truncatula* responds to AHLs produced by the symbiotic bacteria *Sinorhizobium meliloti*, regulating the expression of different defense response proteins such as ROS (Reative Oxygen Species) pathways members and pathogen related proteins (PRs). However, there is not a consensus whether AHLs constitute another class of molecule involved in the plant-bacteria recognition process, but it is reasonable to expect that it could be involved during the first steps of the endophytic colonization by AEDB.

Finally, a well-known class of molecules that is essential for rhizobia and AMF recognition by plants are the lipochitooligosaccharides (LCOs) (D’Haeze and Holsters 2002). Their chemical structure constituted by a backbone of four or five N-acetylglucosamine (GlcNAc) residues, which varies widely among different microorganisms species, is important for the specificity of plant-microorganism interactions (Roche et al. 1991; Dénarié et al. 1996; D’Haeze and Holsters 2002). LCOs recognition by the plant immune response is crucial for the interaction since bacterial mutants defective in LCOs production could no longer associate with their host plants (Dénarié et al. 1996; Oldroyd and Downie 2004). Although the production of LCOs by AEDB has not been reported, this class of bacteria can produce peptidoglycans (PGNs), which share structural similarities with LCOs as they consist of two alternating sugars, GlcNAc and N-acetilmuramic acid (MurNAc) (Pel and Pieterse 2013). PGNs triggered a substantial reprogramming of the plant transcriptome in *Arabidopsis* treated plants, activating similar defense responses as LCOs such as an enhanced expression of immune marker genes (Willmann et al. 2011).

Plant receptors

The recognition process requires that plants perceive and respond to the bacterial signals. The mechanism is mainly mediated by plant receptors that belong to the family of Receptors Like Kinases (RLK), such as Leucine Rich Repeat containing Receptor Like Kinases (LRR-RLKs), Wall Associated Kinases (WAK), Lectin Receptor Like Kinases (LecRLKs), Lys-motif receptors (LysM), among others (Ringli 2010). However, the description of these receptors focus mostly on pathogenicity (Box 1) and, so far, there are just few studies of their involvement in the perception of beneficial microorganisms.

The best LRR-RLK described is Flagellin-Sensitive 2 (FLS2), that recognizes and directly binds flg22, the immunogenic epitope of the PAMP flagellin (Gómez-Gómez and Boller 2000; Zipfel et al. 2004; Melotto et al. 2006). It has been suggested that FLS2 receptors of different plant species can show differences in the recognition of flg epitopes and that this may reflect the evolutionary history of these species and their adaptation to their microbiota (Zipfel et al. 2004). In addition to its involvement in pathogen perception, FLS2 receptors might also signal beneficial associations (Fig. 2). In *Arabidopsis,* FLS2 expression was induced in plants inoculated with the plant growth promoting bacteria *Burkholderia phytofirmans,* however, plant defense responses were not activated (Trdá et al. 2014). On the other way, in *Vitis vinifera* plants inoculated with the same bacteria, the levels of FLS2 receptor (VvFLS2) increased and triggered plant immune responses (Trdá et al. 2014). FLS2 could possibly represent an important receptor in the recognition process of AEDB, bacteria that are essentially flagellated. Important questions to be determined are the role of FLS2 in such a kind of beneficial association, and whether it varies according to the host genotype and the bacterial flagellin, as described for the other systems.

FLS2 function was related with another LRR-RLK, the BAK1 receptor, forming a complex that recognizes the flg22 elicitor (Chinchilla et al. 2007). BAK1 can act as a co-activator of the FLS2 and contribute to disease resistance against the bacterium *Pseudomonas syringae* (Chinchilla et al. 2007; Shan et al. 2008; Roux et al. 2011; Mueller et al. 2012). ArrayExpress gene expression in nodular structures of *L. japonicus,* showed down-regulation of LjBak1 and LjFLS2 (Høgslund et al. 2009). However, the role of the BAK1 receptor in beneficial diazotrophic associations is still unknown (Fig. 2).

A novel subclass of LRR-RLK family, the SHR5 receptor, was identified in sugarcane plants and might have a role in the recognition of AEDB (Fig. 2) (Vinagre et al. 2006). SHR5 expression is down regulated specifically during association of sugarcane plants with beneficial endophytic diazotrophic bacteria such as *G. diazotrophicus, Herbaspirillum spp. and A. brasilense* (Vinagre et al. 2006). Other LRR-RLKs have also been described as critical for recognition of nodulating diazotrophic bacteria, such as Symbiosis Receptor-like Kinase (SYMRK), important in the recognition of beneficial bacteria and fungi (Demchenko et al. 2004; Gherbi et al. 2008; Zhu et al. 2008; Kosuta et al. 2011); Nodulation Receptor Kinase (NORK) and HAR1/NARK, both involved in establishment of nodulation (Fig. 2) (Miyahara et al. 2008; Høgslund et al. 2009; Okamoto et al. 2009; Reid et al. 2011). In sugarcane, 303 ESTs encoding putative LRR-RLKs were found induced by inoculation with *G. diazotrophicus and H. rubrisubalbicans* (Nogueira et al. 2001), suggesting that this gene family might have important roles in signaling the interaction between beneficial diazotrophic bacteria and plants.

WAK receptors have been proposed to recognize oligogalacturonides (Brutus et al. 2010), triggering the immunity activity of plants (He et al. 1998; Sivaguru et al. 2003; Kohorn and Kohorn 2012). It was proposed for symbionts as rhizobium that Nod factors would induce the plant to produce pectate lyase, an enzyme responsible for pectates cleavage in the cell wall (Xie et al. 2012) and WAK receptors could perceive the oligogacturonides compounds released by this cleavage, triggering plant responses (Moscatiello et al. 2012). An important role of WAK in signaling beneficial associations was demonstrated in *Arabidopsis*, where mutant plants for the gene At1g21240 (a member of WAK family), which is induced by the beneficial rhizobacteria *Bacillus subtilis* FB17, showed decreased FB17 colonization (Lakshmanan et al. 2013). The WAK functions in microorganism perception and in cell elongation suggest that these receptors could have dual role in the interaction with diazotrophic bacteria, in especial with plant growth promoting bacteria (Fig. 2).

LysM family of receptors are well characterized as having a role in recognizing molecules such as fungal chitin, bacterial peptidoglycan (PGN), or bacterial nodulation factors (NF), and play functions in symbiosis and immunity (Kaku et al. 2006; Greeff et al. 2012; Gust et al. 2012; Monaghan and Zipfel 2012). During beneficial associations, LysM-RLKs in legumes can have a key role in the recognition of rhizobial Nod factors. In *L. japonicus* and *M. truncatula*, the recognition of Nod factor produced by the associated bacteria *Mesorhizobium loti* depends on

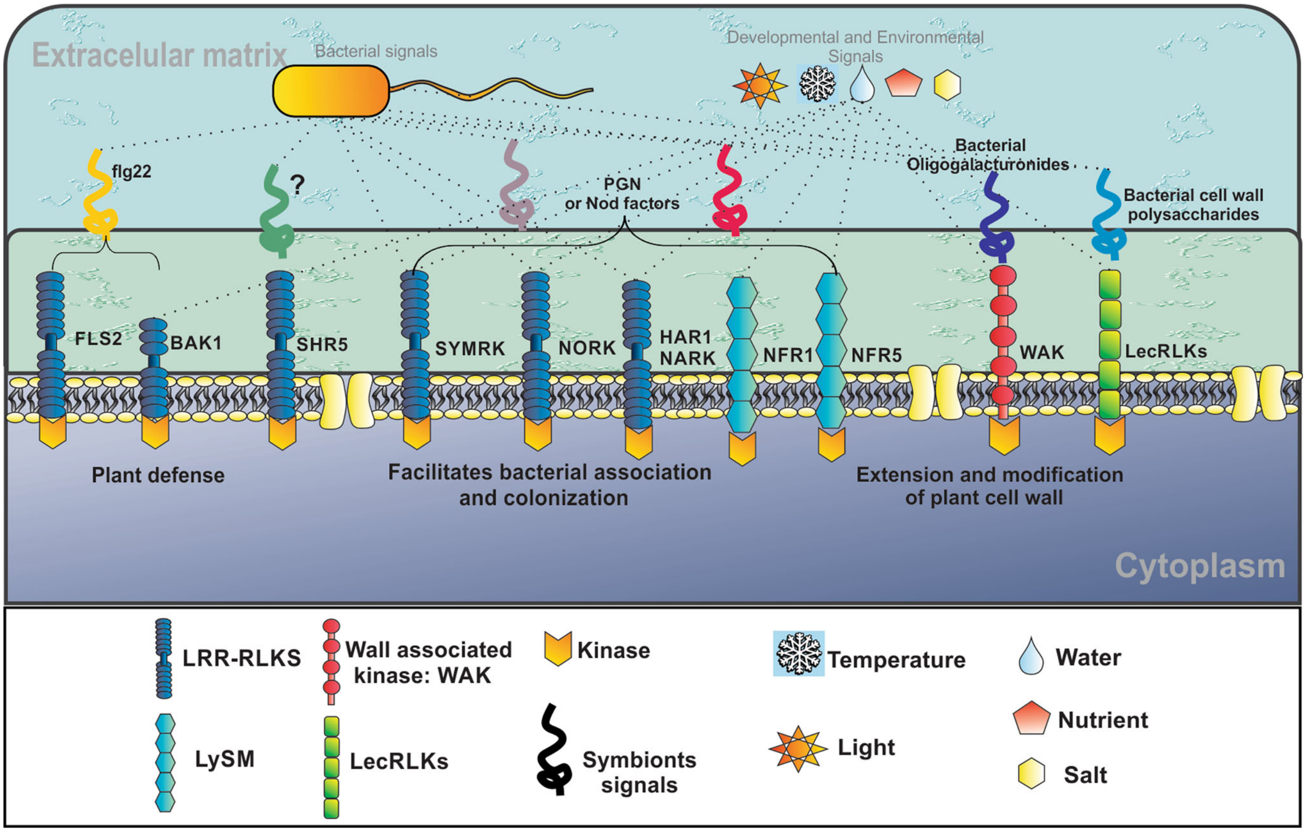

Fig. 2 A model of putative signaling pathways involved in plant recognition of associative and endophytic diazotrophic bacteria. The figure represents various receptor categories in plant cell surface reported to be involved in the recognition of beneficial diazotrophic bacteria effectors and/or of environmental and developmental signals. We hypothesize that during the first contact of AEDB with plants, mechanisms similar to PAMPs are activated and beneficial diazotrophic bacteria might be first recognized as a potential pathogen, activating plant defense pathways. In parallel, some receptors that have the ability to specifically recognize signals of a beneficial bacterium would trigger mechanisms that facilitate bacterial colonization. Possibly, developmental, biotic and abiotic signals can act together regulating responses triggered by plant cell wall receptors to allow the association with beneficial bacteria that will finally culminate in plant adaptations to optimize their growth rates

the LysM-RLK Nod Factor Receptor 1 (NFR1) and NFR5, which may act as heterodimeric Nod factor binding complexes (Radutoiu et al. 2007). Subtle differences in the kinase domain of LysM have been shown to be essential to allow the discrimination between activation of symbiotic processes and activation of plant defense (Gimenez-Ibanez et al. 2009; Willmann et al. 2011). It remains to be determined if LysM receptors have a role in perceiving AEDB signals, such as the PGN. (Fig. 2).

LecRLKs potentially represent a group of receptors that have diverse binding specificities and can bind carbohydrates present in the bacteria cell wall (André et al. 2005; Ringli 2010). However the mechanism of perception of the LecRKs is still unclear, some evidences suggest that adhesins and bacteria cell wall polysaccharides can be the targets of these receptors (van Rhijn et al. 2001). In *Arabidopsis*, some LecRKs were described as possible candidates to interact with the RGD (Arg-Gly-Asp) tripeptide (Gouget et al. 2006), a sequence important for cell adhesion in all multicellular organism. Nonetheless, it is still not well understood the involvement of these receptors in the recognition of beneficial or pathogenic factors (Fig. 2).

Remarkably, several of these cell wall receptors that participate in recognition of microorganisms have also an overlapping role in plant growth and development. In addition, several cell wall receptors were reported to integrate biotic and abiotic environmental signals with plant development, being good candidates to participate in plant-beneficial bacteria signaling, generating adaptive responses of plants to improve growth. For example, BAK1 has a dual role in the perception of brassinosteroids, as well as in participating in the perception of bacterial signals (Clouse 2011; Choudhary et al. 2012). HAR1/NARK are also involved in regulating developmental processes as root growth and cell proliferation (Miyahara et al. 2008; Høgslund et al. 2009; Okamoto et al. 2009; Reid et al. 2011). The LecRKs may have specific roles in cellular morphogenesis (Kijne et al. 1997; Nicholas et al. 1997; Díaz et al. 2000). Also, members of LecRLKs were found up regulated when plants were under saline stress (Joshi et al. 2010). Possibly, developmental, biotic and abiotic signals can act together regulating responses triggered by plant cell wall receptors to allow the association with beneficial bacteria, which in turn can be modulated by the plant in response to a nutritional supplement or in defense against abiotic stress.

Plant epigenetic controls of bacterial recognition

Plant small RNAs (sRNA), as miRNA and small interfering RNA (siRNA), have been described as master regulators of gene expression (Llave et al. 2002; Phillips et al. 2007). They were described as essential for plant growth and development (Vazquez et al. 2004; Kidner 2010) and play important gene-regulatory roles in response to different abiotic stresses (Ding et al. 2009; Zhou et al. 2010). Moreover, sRNAs were also regulated in response to plant–microbe interaction (Navarro et al. 2006; Wang et al. 2009; Thiebaut et al. 2015). Therefore, in addition to the genetic controls, epigenetic pathways are good candidates to be regulating the initial steps of plant-bacteria recognition during AEDB associations.

MiR393, the first shown to be responsive to pathogenic infection, was induced in flg22-elicited *Arabidopsis* seedlings, while its targets, Transporter Inhibitor Response1 (TIR1) and two functional paralogs, were repressed (Navarro et al. 2006). MiR393 was also up-regulated in maize infected with *Rhizoctonia solani Kuhn* (Luo et al. 2014). The inhibition of miR393 targets contributes to antibacterial resistance through repression of auxin signaling pathways (Navarro et al. 2006). In leguminous plants, where auxin also regulates nodule development, miR393 accumulated in soybean roots after 3 h of inoculation with *Bradyrhizobium japonicum* (Subramanian et al. 2008). In contrast, in maize inoculated with the AEDB *H. seropedicae*, the repression of miR393 (Thiebaut et al. 2014) resulted in TIR1 accumulation, releasing ARF mediated auxin-responsive gene expression, finally leading to an attenuation of defense responses (Fig. 3) (Voinnet 2008). MiR160, whose target is also an ARF, was similarly down-regulated in maize-beneficial bacteria association (Thiebaut et al. 2014). The data suggests that an increase of ARF expression could be a mechanism activated during plant recognition of AEDB to help bacterial association by repressing plant defense pathways. In contrast, miR160 was significantly induced in *Arabidopsis* inoculated with the pathogen *P. syringae* pv. tomato (DC3000hrcC), proposing a role of this miRNA regulation on basal defense responses (Fahlgren et al. 2007). The contrasting expression profiles of these miRNAs suggest two possible scenarios in response to plant-bacteria association (Fig. 3).

The involvement of up-regulation of copper related miRNAs (Cu-miRNAs) was also highlighted in the assistance of AEDB colonization (Thiebaut et al. 2014). Since their down-regulated targets have a role in cooper homeostasis and in defense pathways against pathogenic microorganisms, the repression of Cu-miRNAs could possibly facilitate the plant-endophytic diazotrophic bacteria association by the attenuation of defense mechanisms. For instance, the induction of miR397 lead to down-regulation of laccase, which mediates the polymerization of phenolic compounds and cell wall reinforcement that represent an important defense mechanism by prohibiting the entrance of microbes into plant (Whetten and Sederoff 1995; Constabel and Ryan 1998). Therefore, miR397 was repressed in cotton infected with the pathogen *Verticillium dahlia* Kleb (Yin et al. 2012). In contrast, miR397 was induced in the legume-rhizobium interaction (De Luis et al. 2012), as well as in *H. seropedicae* and *A. brasilense* association with maize (Thiebaut et al. 2014). It can suggest that plants could sense the diverse microorganisms and trigger the miRNA regulation accordingly (Fig. 3).

Repression of miR482, which targets Nucleotide Binding Site-Leucine Rich Repeat receptors (NBS-LRR) through secondary siRNA, was induced upon pathogenic infection in tomato (Shivaprasad et al. 2012), implying that defense responses were activated by increase of NBS-LRR expression. In *Rhizobium*-soybean interaction, miR482 was up-regulated (Subramanian et al. 2008), in contrast, it was not identified in maize inoculated with *H. seropedicae.* MiR482 was also involved in production of secondary siRNA, which regulate other defense-related proteins (Shivaprasad et al. 2012), indicating a possible role of siRNAs in plant–microbe interaction (Fig. 3). Nevertheless, siRNAs that aligned with repetitive sequences, which may silence transposable elements, were up-regulated in maize inoculated with AEDB (Thiebaut et al. 2014), suggesting that siRNA could be also silencing genes by methylation during recognition of the beneficial diazotrophic bacteria (Fig. 3).

Conclusions and future outlook

The establishment of a beneficial interaction with associative and endophytic diazotrophic bacteria can bring several adaptive responses to plants that will culminate with an improvement in growth at their surrounding environment. There is an increasing amount of studies supporting the benefits such type of association can bring to plants and to a more sustainable agriculture. As the

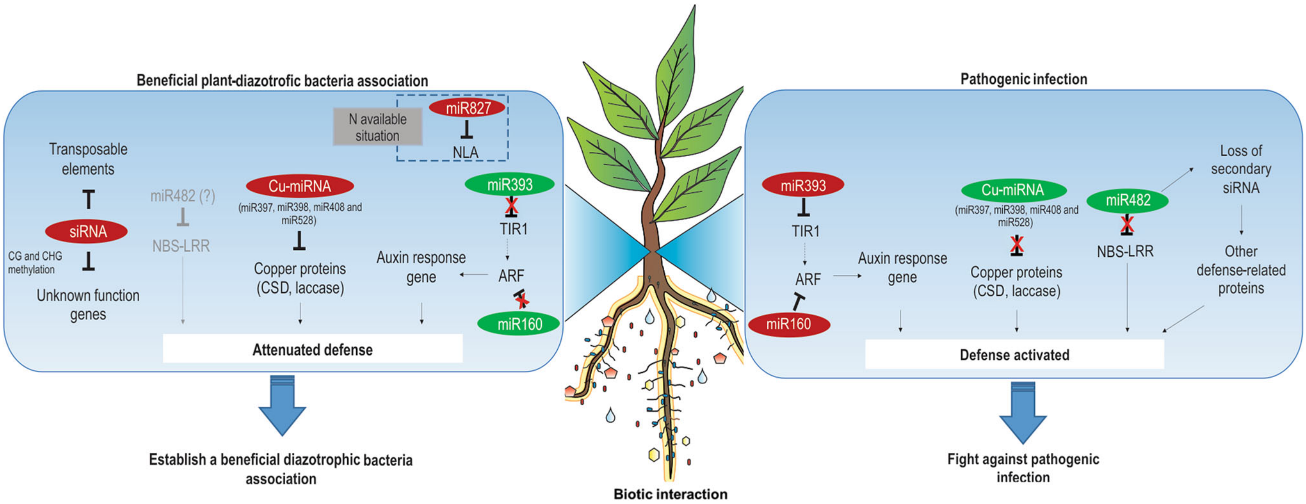

Fig. 3 Two scenarios of sRNA regulation in plant-bacteria association. On the *left*, plants can modulated the miRNA-target regulation to facilitate the association with diazotrophic bacteria, inhibiting defense pathways. On the *right*, plants can recognize the pathogen, for example, by recognition of elicitor flg22, and trigging regulation by sRNA, finally leading to defense responses. Red balloon: sRNA are up-regulated; *Green balloon* sRNA are down-regulated. In *gray* is a hypothetical regulation. *CSD* Copper Superoxide Dismutase

understanding of the genetic and biochemical mechanisms regulating such plant–microbe type of interaction is moving fast, it brings novel fascinating questions to be answered. It becomes clear that the initial steps of perception and recognition of the bacteria as beneficial is crucial to determine the outcome of the association.

One still intriguing question is whether and how plants use different signaling pathways to recognize and distinguish beneficial from pathogenic bacteria. As discussed in this review, the structure of the signals and receptors are very similar among both types of interaction, as well as the regulatory miRNAs and the plant signaling responses activated by pathogenic and beneficial interactions share some overlap. We can speculate that during the first contact with plants, mechanisms similar to PAMPs are activated and beneficial diazotrophic bacteria might be first recognized as a potential pathogen, activating plant defense pathways. In parallel, some receptors that have the ability to specifically recognize signals of a beneficial bacterium would trigger mechanisms that suppress some defense responses to allow bacterial colonization.

At this point a second fascinating question emerges: are these bacteria always perceived by plants as "beneficial and necessary"? Although there are a lot of beneficial outcomes from associations with AEDB, they do not always establish beneficial associations with plants. Environmental conditions, such as water availability and nutrient supply in soil, may regulate the establishment of a beneficial association. *H. rubrisubalbicans* is pathogenic to some sugarcane cultivars. Thus the benefits of the AEDB to the plant are dependent on plant genetic factors as well as environmental conditions. As discussed previously, the homeostasis of the whole "soil–rhizosphere–rhizoplane–endophytes–plant" system should recruit different levels of regulation, to generate plants better adapted to the environment. Therefore it is reasonable to expect that the recognition between the parties might represent a dynamic key point of regulation, that perceives the genetic and environmental conditions and coordinates the transduced responses with pathways that govern the efficiency and/or the type of beneficial outputs provided by the interaction.

To fully understand how plants allow the entry of AEDB to establish a beneficial association with them, it is necessary to expand the knowledge of the key regulators involved. Different bacterial signals, plant receptors and miRNA targets seem to regulate this type of plant–microbe interaction, it is now necessary to carry on functional analyses to elucidate their role in the interaction. These genes could be used as tools to assist breeding programs to develop cultivars more efficient in association with AEDB, leading to yield improvement and more sustainable agriculture practices.

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