A review of the effects of soil organisms on plant hormone signalling pathways

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A B S T R A C T

Plants interact with a large number of soil organisms. For a long time, these interactions have been the research area of soil ecologists and trophic relationships and physico-chemical modifications of the soil matrix were generally proposed as mechanisms underlying plant-soil organism interactions. However, some specific symbioses and diseases have been well characterized at the molecular level by plant biologists and microbiologists. These interactions involve a physical contact between soil organism and plant. They are mediated through signal molecules that play upon the different plant hormonal signalling pathways, leading to modifications in plant development and defence. Nowadays, the role of signal molecules emerges as an important feature of interactions between plants and free-living soil organisms. In this review we discuss genetic and physiological evidences of hormone signalling involvement in plant response to physically associated but also free-living soil organisms, for very different taxa ranging from the micrometer to the centimetre scales. The same hormone signalling pathways seems to be activated by very different kinds of soil organisms such as bacteria, nematodes, collembola and even earthworms, with common consequences on plant growth, development and defence. Plant hormonal homeostasis appears to be the corner stone to understand and predict the issue of the multiple interactions that plants entertain with the community of soil organisms.

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1. Introduction

Soil organisms, defined as organisms spending one part of their life cycle in the soil and interacting with plant belowground organs, coevolved with plants, becoming more or less strong selective constraints. The physical association between plants and soil organisms such as arbuscular-mycorrhiza (AM), ecto-mycorrhiza (ECM) and pathogens has led to co-evolution sensu stricto, revealed by a molecular dialog between plant and soil organism (Desbrosses and Stougaard, 2011; Robert-Seilaniantz et al., 2011). These organisms generally dependent on plant for their resources and their habitat could even hijack plant morphogenesis to create their own niche (Odling-Smee et al., 1996). The production of elicitor specifics of the host plant is required for the establishment of a compatible association. Free-living organisms without physical contact with plants have developed diffuse co-evolution with plants (Janzen, 1980), since biotic and abiotic environment of the two partners influences their relationships. These organisms are less dependent on plants for their survival and reproduction. In this case, the release of non-specific diffusive signal molecules initiate the dialog between plant and free-living organisms.

Up to now, knowledge about soil organism impact on plants is generally viewed as a collection of studies involving a plant and one specific soil organism well known by a restricted number of specialists. Few papers consider analogies in plant response to very different soil organisms (Grunewald et al., 2009b; Hause and Schaarschmidt, 2009; Lohar et al., 2004). However, we will show that the development of the use of mutants or -omic methods reveals common features in plant response to a diversity of soil organisms such as bacteria, fungi, but also protozoa, nematodes and earthworm. Here, we propose the hypothesis that the diversity of plant-soil organism interactions is mainly based on signal molecules which impact a restricted number of plant signalling pathways. Our review aims at describing a general emerging framework for soil organism effects on plant signalling pathways, covering a large spectrum of taxa from microorganisms to micro-, meso- and macro-fauna, physically associated or not with plants. Interactions between plants and leaf pathogens have been
3. Signal molecules and hormone signalling networks involved in plant development and defence

Genetic evidences of the involvement of hormone signalling in the response of plants to soil organisms are generally provided using loss-of-function or gain-of-function mutants affected in one or more gene encoding proteins involved in hormone signalling pathway. Using this strategy, several steps from signal perception to adaptation have been identified (Fig. 1): after (1) the perception of biochemical compounds, (2) these signals are transduced in cells and modulate gene expression; during the signalling step sensu stricto different response mechanisms are activated leading to (3) the modification of plant development or (4) defence mechanisms, with consequences on plant growth; (5) feedback loops on the same or other signalling pathways ensure a coordinated response. These different steps are detailed successively in the following sub-sections.

3.1. Signals

Signal molecules are molecules with strong effects on organism physiology despite their presence at very low concentration in the environment (Zhuang et al., 2013). They are generally associated with qualitative changes (e.g. development and/or defence), which could result in quantitative changes (e.g. growth). They differ from nutrients which are constitutive of biomass, generally present at relatively high concentration and responsible for quantitative changes, not always due to qualitative ones. The main signal molecules in plants are hormones, such as auxins (IAA), cytokinins (CK), gibberellins (GA), abscisic acid (ABA), ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) synthesized in plant cells. Several soil microorganisms exhibit their own biosynthetic pathway for the major plant hormones, which can be produced in culture media (Frankenberger and Arshad, 1995; Persello-Cartieaux et al., 2003; Robert-Seilaniantz et al., 2011). Soil organisms can also produce phytoxins like coronatine, a mimic of jasmonic acid (JA)-isoleucine, but also signal molecules recognized as elicitors of plant defence such as chitin, flagellin and lipopolysaccharide (Bakker et al., 2007; Pieterse et al., 2009). Some of these signals are gaseous molecules, generally volatile organic compounds (VOCs) (Desbrosses et al., 2012; Ping and Boland, 2004). Some soil organisms can degrade plant hormones or their precursors; for example, bacteria are able to reduce ET concentration through an 1-Amino cyclopropane-1-carboxylic acid deaminase (AcdS) activity, which degrades the plant ET precursor 1-amino cyclopropane-1-carboxylic acid (ACC) in α-ketobutyrate and ammonia (Desbrosses et al., 2012; Glick, 2005). In a lesser extent, signal molecules could also be components of the plant released during interaction with pathogens (Hernández-Mata et al., 2010).

3.2. Hormonal signalling networks

After signal perception by the plant, the transfer of this signal is relayed by hormonal signalling pathways under the influence of major plant hormones (Fig. 2). Here we will provide background elements to the neophyte, in the aim to get straight to the point in the following sections.

3.2.1. Auxin

Indole acetic acid (IAA) signalling relies on the influx of IAA into the cells through IAA influx carrier (AUX1) proteins. IAA stabilizes the interaction between the F-Box transport inhibitor response 1/auxin signalling F-box (TIR1/AFB) receptor located into the Skp Cullin F-Box (SCF) SCF[TIR1]/AFB β3 ubiquitin ligase complex and the negative regulator of IAA responsive genes auxin/indole-3-acetic
acid (AUX/IAA) proteins. The association between AUX/IAA and F-box protein TIR1/AFB activates the E3 ligase leading to AUX/IAA ubiquitination and degradation through 26S proteasome. Degradation of AUX/IAA results in derepression of Auxin Response Factor (ARF) transcription factor and consequent induction of expression of early IAA responsive genes. Among these genes rapidly induced in response to an IAA stimulus, the most characterized are three gene families: Aux/IAA, GH3 and small auxin-up RNAs (SAURs) (Cohen and Gray, 2007) (Fig. 2A).

3.2.2. Cytokinins

Cytokinins (CK) are perceived by membrane located histidine kinase receptors (AHK) such as CRE1. Binding of CK leads to the autophosphorylation of AHK. These different pathways are detailed successively in Section 3.2. Adapted from the KEGG PATHWAY database (http://www.genome.jp/kegg/pathway.html) (Kanehisa et al., 2012). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
transferred via a histidine phospho-transfer protein (AHP) from the cytoplasm to type-B response regulators (B-ARRs) in the nucleus. Type-B ARR bind to DNA and activate the expression of target genes including type-A-ARRS regulators, involved in the negative feedback of CK on its own signalling pathway (Heyl et al., 2007) (Fig. 2B).

### 3.2.3. Gibberellic acid

Gibberellic acid (GA) signalling is activated when GA level crosses a given threshold. Then GA binds to the nuclear localized gibberellin-insensitive dwarf 1 (GID1) protein. This promotes the association of GID1 with DELLA proteins and lead to the recognition of slender1 SLR1/DELLA proteins by the GID2 F-box containing SCF^GID2^3 ubiquitin ligase complex, resulting in its ubiquitination and degradation by the 26S proteasome. Degradation of DELLA proteins leads to the activation of transcription factors (TF) such as PIF3/4, which positively regulate GA-signalling pathway (Thomas and Hedden, 2007) (Fig. 2C).

### 3.2.4. Abscissic acid

Abscisic acid (ABA) binds to pyrabactin resistance (PYR) or pyrabactin resistance–like (PYLs) proteins causing a conformational change enabling the binding to and inhibition of the phosphatase activity of serine/threonine phosphatase 2Cs (PP2Cs) proteins. As a consequence, SNF1-related protein kinase 2s (SnRK2s) are released from inhibition and regulate key targets of ABA signalling pathway such as bZIP protein ABA-responsive elements binding factor (ABFs) which interacts with ABA responsive elements (ABREs) (Raghavendra et al., 2010) (Fig. 2D).

### 3.2.5. Ethylene

Ethylene (ET) signalling pathway relies on the perception of ET by endoplasmic reticulum membrane histidine kinase receptors (ETRs). In the absence of ET, ETRs maintain the negative regulatory role of CTR1. Upon ET perception, CTR1 is thus inactivated by ET resulting in derepression of ethylene insensitive protein 2 (EIN2).

In the nucleus, an ET-dependent transcriptional cascade occurs. Members of EIN3/EIN3-like (EIL) transcription factor family bind to ethylene responsive element–binding protein (ERE1/2) transcription factors. In the absence of ET, EIN3 is targeted for ubiquitination by EIN3-binding F-box protein 1 and 2 (EIBF1/2) parts of SCF^EIBF1/EIBF2^ complexes and degraded by the 26S proteasome (Bisson and Groth, 2012; Vandenbussche et al., 2007) (Fig. 2E).

### 3.2.6. Jasmonic acid

Jasmonic acid (JA) signalling involves the adenylation of JA by JA conjugate synthase (JAR1) protein, leading to the conjugation of the oxylipin JA with amino acids (generally isoleucine). JA-Ile acts as molecular glue between the F-box protein COI1 in the E3 ubiquitin ligase SCF^COI1^ complex and the negative regulator of JA-signalling the jasmonate ZIM domain (JAZ) proteins. Thus JAZ proteins are ubiquitinated and subsequently degraded through the 26S proteasome. This results in the activation of JA-responsive genes through the action of transcription factors such as MYC2 (Wasternack, 2007) (Fig. 2F).

### 3.2.7. Salicylic acid

Salicylic acid (SA) mediates change in cellular red-ox potential resulting in the reduction of the non-expressor of PR1 protein (NPR1) oligomer to its active monomeric form. Monomeric NPR1 is then translocated into the nucleus where it functions as a transcriptional co-activator of SA-responsive genes, such as PR-1, by enhancing the binding of TGA basic leucine zipper (bZIP) transcription factors to SA-responsive promoter elements. The name “TGA” refers to TGACG-, the sequence reported to be the preferred substrate for TGA factors (Robert-Seilanianz et al., 2011) (Fig. 2G).

### 3.3. Development

Plant development is highly dependent on hormone signalling. Modifications of several plant developmental processes in response to soil organisms have been largely reported for shoot:root ratio, root system structure via root elongation, lateral root emission and elongation, shoot morphology via leaf area, mass per unit area, floral stem height, grain yield, seedling emergence and vigour (Blouin et al., 2007; Grunewald et al., 2009a; Jana et al., 2010). Interaction with few soil organisms can also lead to de novo organogenesis of specific organs essentials for the interactions. This includes the formation or root nodules in the presence of Rhizobium, but also galls due to nematodes or bacterial infections. These modifications of plant development and morphology can be beneficial (e.g. change of root morphology to optimize nutrient uptake) or detrimental (e.g. formation of feeding sites) to the plant.

### 3.4. Defence

Plant defence relies on the detection of pathogen conserved molecules called pathogen-associated molecular patterns (PAMPs) by plant protein recognition receptors (PRRs). This leads to the activation of defence mechanisms known as PAMP-triggered immunity (PTI). Some pathogens secrete effector proteins that deregulate PTI allowing effective plant infection. This mechanism is known as effector-triggered susceptibility (ETS) and results in parasitic relationship establishment. In response to this kind of attack, plant resistance (R) proteins recognize effectors, thus activating effector-triggered immunity (ETI). Upon the recognition of these elicitors, local and systemic responses are activated in the plant. One of these mechanisms of resistance is associated with the SA-signalling pathway that leads to expression of pathogenesis-related (PR) proteins which contribute to immunity. This mechanism is induced upon plant challenge by avirulent pathogen or upon restricted infection by virulent pathogen (Fig. 3). Systemic activation of this mechanism confers long-lasting protection against a broad-spectrum of pathogens and is referred as Systemic Acquired Resistance (SAR) (reviewed by Glazebrook, 2005). Other plant defence mechanisms involving the JA- and ET-signalling pathways can lead to the expression of defence related genes such as PDF1.2 or PR-4 encoding a Hevein-like protein (HEL). This mechanism is triggered by non-pathogenic rhizobacteria and will also confer a broad-spectrum protection against pathogens. It is referred to as induced systemic resistance (ISR) (Fig. 3). SAR and ISR are effective for different pathogen lifestyles. Whereas SAR is generally considered efficient against biotrophic pathogen (i.e. feed on living host tissues), ISR is generally efficient against necrotrophs pathogens (i.e. first destroy host cell, then feed on the remains) (Glazebrook, 2005; Pieterse et al., 2009). Defence and growth are not independent processes: there is a negative trade-off between these two aspects of plant metabolism due to the limited energetic budget of an organism (Denancé et al., 2013; Kazan and Manners, 2009) (Fig. 3). In Sections 4 and 5, we will only discuss the “direct” effects on plant development and not “indirect” effects due to this trade-off.

### 3.5. Crosstalk between hormone signalling pathways

The previously described hormone signalling pathways are not independent from each other. Several regulatory feedback loops exist, which are responsible for coordination between development and defence, resulting in a growth rate in adequacy with the biotic and abiotic environment. IAA crosstalk with ET is essential in several developmental processes: they act synergistically to
control root elongation and root hair formation and antagonistically in lateral root formation and hypocotyls elongation (Muday et al., 2012). IAA and ET also interact with CK signalling. In the shoot apical meristem, IAA and CK act synergistically, as IAA maintains high CK levels in the centre of the meristem. Conversely, high CK levels in the root tip restrict IAA-signalling allowing cell differentiation (Durbak et al., 2012). ET signalling through EIN3 suppresses the expression of A-ARR genes thus repressing CK signalling. Conversely, CK increases ET biosynthesis through the stabilisation of 1-aminocyclopropane-1-carboxylate synthase 5 (ACS5) and ACS9 involved in ET-biosynthesis. Crosstalk between ET- and CK-signalling might consequently affect plant growth processes such as root growth (El-Showk et al., 2013; O’Brien and Benkova, 2013).

Concerning plant immunity, there is a strong cross-talk and antagonism between SA- (associated with SAR) and JA/ET-signalling (associated with ISR), where NPR1 protein plays an important role (Pieterse et al., 2009; Pieterse and Van Loon, 2004; van Loon et al., 2006). Even if SA and ET/JA are the basic hormones associated with SAR and ISR, respectively, they are more and more evidence that other plant hormones (IAA, GA, ABA and CK) are important modulators of plant defence against pathogens (Grant and Jones, 2009; Pieterse et al., 2009; Robert-Seilaniantz et al., 2007, 2011). In the following sections we will discuss the involvement of plant hormone signalling pathways in the effect of different classes of soil organisms on plant development and immunity.

4. Soil organisms establishing a physical contact with plant roots

This kind of organism has been well studied, since it is easy to attribute modification in plant development and defence to the interacting organism. Here, we provide essential and synthetic information on these well-known interactions, analyzed in details in other articles.

4.1. Root associated bacteria

4.1.1. Symbiotic bacteria

Legumes are capable of symbiotic association with Rhizobium. This interaction results in visual symptoms, with de novo organ formation, called nitrogen-fixing nodules. These nodules fix atmospheric N₂, supplying plants with nitrogen; in counterpart, plants provide a carbon source for Rhizobium strain. This very specific interaction leads to major developmental and metabolic changes for both organisms. Bacterial infection is initiated by the penetration of Rhizobium by cracks in the root epidermis or through root hair curls. Signal molecules commonly called Nod factors, produced by Rhizobium, initiate a molecular dialogue (Fig. 1) between the host plant and the compatible strain (Desbrosses and Stougaard, 2011; Ferguson and Mathesius, 2003; Oldroyd and Downie, 2008). These Nod factors are perceived by receptors inserted in the plasma membrane of plant cell (Oldroyd and Downie, 2008) leading to the progression of Rhizobium towards the root cortex. In response to Nod factors two processes are initiated: (i) an organogenic process leading to the development of the nodule (Fig. 3), and (ii) an infection process mediating bacterial colonization (Oldroyd and Downie, 2008). Organogenesis requires the involvement of plant hormones (Desbrosses and Stougaard, 2011; Ferguson and Mathesius, 2003; Oldroyd and Downie, 2008); this demonstrates the importance of co-evolution between plant and symbiont in symbiont niche construction (Odling-Smee et al., 1996). CK and IAA are of first importance in nodule development. On L. japonicus, a gain-of-function on the snf locus leads to spontaneous development of white Rhizobium-free root nodules in the absence of Mesorhizobium loti (Tirichine et al., 2006). The gene snf2 has been identified as an allele of a Lotus Histidine Kinase (LHK1), which encodes a protein closely related to the A. thaliana CK receptor CRE1 (Tirichine et al., 2007). The involvement of CK signalling in nodule formation was confirmed by studies on L. japonicus loss-of-function mutation of LHK1: both lhk1 and hit1 mutants are insensitive to CK and exhibit a nodulation deficient phenotype (Murray et al., 2007; Tirichine et al., 2007). Similarly, in M. truncatula, RNAi mediated down-regulation of MtCRE1 and the use of loss-of-function cre1 mutant leads to
CK insensitivity and strongly reduced nodulation (Gonzalez-Rizzo et al., 2006; Plet et al., 2011). Moreover, CK signalling is involved in the regulation of IAA accumulation during nodule development. Indeed, in loss-of-function *cre1* mutant, the inhibition of polar IAA transport by *Rhizobium* is suppressed and an accumulation of PIN auxin efflux carrier is observed as compared to WT (Plet et al., 2011). CK signalling acts as a regulatory knob, integrating positive plant and bacterial cues to control legume nodule organogenesis (Gonzalez-Rizzo et al., 2006; Plet et al., 2011; Suzuki et al., 2013).

The formation of nitrogen-fixing nodules is an organogenetic process, but also an infection process. The regulation of nodulation by stress related hormone (ET, JA, SA and ABA) has been proposed as a mechanism to balance nodulation level with the overall plant health. Bacterial nitrogen fixation represents a significant carbon drain, and in some situations, nitrogen availability is not a critical factor limiting plant growth (Morgan et al., 2005; Oldroyd and Downie, 2008). Involvement of ET-signalling in the control of nodulation was fully demonstrated with *M. truncatula* loss-of-function *skl1* (the *M. truncatula* homolog of *A. thaliana* *EIN2*) and *L. japonicus* over-expressing the dominant *etr1-I* allele of *A. thaliana* mutant. These hypernodulating mutants are insensitive to exogenous application of ET or ACC, the immediate precursor of ET. These treatments inhibit nodulation in WT but not in *skl1* or *let* mutant, validating the involvement of ET signalling in nodulation control (Lohar et al., 2009; Varma Penmetsa et al., 2008). Moreover, ET signalling has been shown to interact with other hormones signalling, namely JA and GA (Ferguson et al., 2011; Sun et al., 2006). Overexpression of the gain-of-function SLEEPY1 mutation in *L. japonicus* resulted in a reduced nodule number (Maekawa et al., 2009). SLEEPY1 (SLY1) has a function as positive regulator of GA signalling by interacting with the negative regulator of GA signalling (DELLA proteins). These results were confirmed in *Pisum sativum* loss-of-function cry-s mutant that lacks DELLA proteins and thereby shows a constitutive GA-signalling. In this mutant, a reduced number of nodules was observed (Ferguson et al., 2011).

Involvement of ABA was demonstrated using transgenic line of *M. truncatula* over-expressing the dominant *abi1-I* allele of *A. thaliana* leading to the genetic inhibition of ABA signalling. This genetic inhibition of ABA signalling results in a hypernodulation (Ding et al., 2008). Transgenic lines of *L. japonicus* and *M. truncatula*, unable to accumulate SA due to ecotopic expression of the bacterial nahG gene encoding a salicylate hydrolase, were subject to an increase in infection and nodule number when compared to WT (Stacey et al., 2006). These studies validate the involvement of stress related plant hormone signalling pathways in the control of nodulation.

4.1.2. Pathogenic bacteria

In contrast to *Rhizobium* whose interaction with plants leads to a symbiotic association, other bacteria such as *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* are pathogenic and their association with plant results in the development of tumours or galls (Fig. 3). Whereas *Agrobacterium*-mediated transformation of plant cells has been extensively studied, their impact on plant development and defence is still limited. Tumour or gall formation induced by *Agrobacterium* strains involves transfer of T-DNA from bacteria into the genome of host infected cells. This T-DNA possesses several genes encoding proteins involved in IAA and CK biosynthesis pathway. These proteins lead to an overproduction of IAA and CK by the transformed plant cells, resulting in the formation of tumour or gall (Spaepen et al., 2007). In addition to the transfer of genes encoding proteins involved in IAA-biosynthesis, a plant functional IAA-signalling pathway is also required as shown by the altered tumour formation in response to *A. rhizogenes* and *A. tumefaciens* in loss-of-function mutants impaired in IAA-signalling (*axr1-I, axr1-12 and axr2*) (Lincoln et al., 1992). The transcriptome of *A. thaliana* in response to *A. tumefaciens* CS8 reveals several genes involved in IAA-signalling in the early steps of the infection, followed by the activation of genes involved in IAA- and ET-signalling after T-DNA transfer (Lee et al., 2009). Besides the involvement of hormone signalling in tumour or gall formation, *Agrobacterium* strains also interact with plant defences. In virus-induced gene silencing of *Nicotiana benthamiana* genes *ICS* (SA-biosynthesis), *NPR1* and *SABP2* (SA-signalling), susceptibility against *A. tumefaciens* is increased (Anand et al., 2008). In *A. thaliana*, *A. tumefaciens* infectivity was enhanced in mutants characterized by constitutive low levels of SA. However, opposite results were found for SA-signalling involvement since *A. tumefaciens* infectivity was reduced in loss-of-function *npr1* SA-signalling mutants and *cpr5* mutant which overaccumulate SA (Lee et al., 2009; Yuan et al., 2007). Even if the interaction of *Agrobacterium* strains with host defence is still elusive, these results suggest that SA-signalling plays a role, likely by direct interaction with the pathogen (Anand et al., 2008; Lee et al., 2009; Yuan et al., 2007).

4.2. Root associated fungus

In contrast, with the symbiosis with *Rhizobium*, limited to the Fabaceae family, a large number of plant taxa are able to establish symbiotic association with mycorrhizal fungi. These interactions involve plants and soil-borne fungus.

4.2.1. Arbuscular and ecto-mycorrhizae

Fungal symbioses are often referred to as arbuscular mycorrhizae (AM) and ecto-mycorrhizace (ECM). They represent the most widespread symbiotic association among plants and affect about 82% of the plants in natural, agricultural and forest ecosystems (Brundrett, 2002). This “extension” of roots helps the plant in taking up water and minerals, notably phosphate (Brundrett, 2002). Enhancement of lateral root emergence and elongation by AM or ECM prior to symbiosis establishment can also occur without mycorrhizal plants such as *A. thaliana* (Felen et al., 2009; Spilvallo et al., 2009). This suggests that plant development response to AM or ECM does not depend on plant-fungus compatibility and mycorrhiza establishment. Changes in plant development in the presence of AM or ECM are dependent on plant hormone signalling. Hence, analysis of poplar transcriptome during interaction with the ECM *Laccaria bicolor* reveals the involvement of several components of polar IAA transport (*PtaPIN* and *PtaAUX* genes), IAA conjugation (*PtaGH3* genes) and IAA signalling (*PtaIAA* genes). The involvement of IAA signalling was confirmed by the loss of lateral root stimulation in *A. thaliana* loss-of-function *tir1afb1,2,3* and *slr1* mutants (Felen et al., 2009). In a similar way, modulation of root morphogenesis of *A. thaliana* by *Tuber borchii* requires IAA signalling but also ET (Spilvallo et al., 2009). As compared to WT, primary root growth was not inhibited by *T. borchii* in the *Arabidopsis* IAA transport mutant aux1-7 and root branching was less affected in the ET-insensitive mutant ein2-LH. Furthermore, the double loss-of-function aux1-7ein2-LH mutant displayed reduced sensitivity to fungus-induced primary root shortening and branching (Spilvallo et al., 2009). IAA signalling is also required for initiation of the symbiosis between the AM *Glomus intraradices* and *Solanum lycopersicum*. Presymbiotic root branching was analyzed in loss-of-function *pc* and *dgt* mutants, affected, respectively, in IAA transport and signalling. Root branching was stimulated in *pc* but not in *dgt* roots and both *pc* and *dgt* roots were poorly colonized (Hanlon and Coenen, 2011). These studies also underline that modification of plant morphology by ECM or AM does not require a physical contact and was due to diffusive molecules, which interact with IAA signalling. Therefore, changes in hormone homeostasis occur prior to mycorrhiza formation (Felen et al., 2009; Hanlon and Coenen, 2011; Spilvallo et al., 2009). Like *Rhizobium*, symbiosis with ECM or AM involves an infection process. However, the modulation of
4.2.2. Endophytic fungus

The endophytic fungus Piriformospora indica is known to colonize roots of a wide range of plants, including *A. thaliana*, and inversely in ECM and AM they could live independently of the plant (Fig. 3). In comparison to the previously described organisms, little is known about the involvement of hormone signalling in the effect of this endophytic fungus on plant development. It seems to be independent of changes in IAA levels or the expression of IAA-related genes (*Lee et al., 2011; Vadassery et al., 2008*). However, CK-biosynthesis and signalling is required as shown by the changes in GUS activity in the roots of *A. thaliana* transgenic *ARR5::uidA* lines colonized by *P. indica*. Moreover, growth promotion of *A. thaliana* by *P. indica* is lost in loss-of-function *at ipt1,3,5,7* and *cre1ahk2* mutant (*Vadassery et al., 2008*) affected in CK-biosynthesis and signalling, respectively. As far as infection and defence mechanisms are concerned, this endophytic fungus shows a biotrophic followed by a necrotrophic growth phase characterized by cell death-associated colonization (*Jacobs et al., 2011; Lahrmann and Zuccaro, 2012; Schäfer et al., 2009*). These steps are highly dependent on plant hormone signalling. Indeed, growth promotion is lost in *A. thaliana* loss-of-function *etr1, ein2* and *ein3eil1* ET-signalling mutants. Moreover, the roots of these mutants are more colonized as compared to WT (*Camehl et al., 2010*). Importance of ET-signalling is confirmed by the overexpressing ERF1 transgenic line which shows a strongly reduced colonization and abolishment of plant growth promotion (*Camehl and Oelmüller, 2010*). These results suggest the importance of ET-signalling as a feedback mechanism to balance beneficial and non-beneficial effects of the fungus (*Camehl and Oelmüller, 2010; Camehl et al., 2010*). Accumulating clues suggest that this fungus does not evade plant detection but rather hijack plant immunity through the emission of microbe-associated molecular patterns (MAMPs) (*Jacobs et al., 2011; Lahrmann and Zuccaro, 2012; Schäfer et al., 2009*). Hijack of plant innate immunity is compromised in loss-of-function *jin1-1* and *jar1-1* JA-signalling mutants, since roots are less colonized as compared to WT. Moreover, GA-signalling mutant with a loss-of-function for all five DELLA proteins is less colonized than WT at the beginning of the infection, but more colonized in the later stage of the infection. Conversely, loss-of-function *gai*-*6* mutant affected in GA-biosynthesis shows a higher degree of colonization. These results suggest that *P. indica* suppress plant innate immunity and recruits GA-signalling in the cell-death associated colonization stage (*Jacobs et al., 2011; Schäfer et al., 2009*).

4.2.3. Pathogenic fungus

Pathogenic fungus such as *Fusarium oxysporum* interacts also with hormone signalling during the different stages of the colonization process. A comprehensive study of changes in hormone signalling during the different stages of colonization showed that IAA and JA play an important role. *A. thaliana* loss-of-function *coi1* mutant affected in JA-signalling is resistant to *F. oxysporum* (*Thatcher et al., 2009*). However, JA-biosynthesis is not involved, as the severity of the disease remains the same in loss-of-function mutants impaired in JA-biosynthesis (*Thatcher et al., 2009*). Disease severity was diminished in loss-of-function *axr1, axr2, axr3* and *sgt1b* IAA-signalling mutants but also in loss-of-function *axr4, aux1, pin2/etr1* and *big/doc* IAA-transport mutants (*Kidd et al., 2011*). These results suggest that *F. oxysporum* hijacks defence mechanism and exploits IAA-signalling and transport to infect plant roots.

4.3. Associated microfauna

Soil animals with a size up to 100 μm belong to microfauna. The main taxonomic groups are Nematoda and Protozoa (*Decaëns, 2010*). Effects of these organisms on plant development and immunity range from beneficial to deleterious. Some of these small soil animals are physically associated with plant roots, like nematodes and pathogenic protozoa (Fig. 3).

4.3.1. Plant parasitic nematodes

Root colonization by nematodes relies on their ability to produce many effectors which can have diverse functions. Some are involved in the suppression of plant defence, while others can specifically interact with plant signalling pathways to promote the formation of nematode feeding sites (*Haegeman et al., 2012*). This feeding site allows the development of either a gall in plant tissues, or a cyst located outside root tissues, derived from the body of adult nematode. The new generation then emerges from these structures (*Barker et al., 1998*). The modification of root morphology induced by nematodes requires components of host’s IAA-signalling and transport pathways (*Grunewald et al., 2009b; Kazan, 2013*). Indeed, nematodes infectivity is compromised on *A. thaliana* loss-of-function *pin* mutants with a deficient IAA transport (*Grunewald et al., 2009a*). Moreover, a decrease in root colonization by *Heterodera schachtii* has been observed in both aux1*lasx3* double mutant and aux1*lasx1ax2lasx3* quadruple mutant affected in IAA transport and in the effector protein Hs1919C07 which physically interacts with *A. thaliana* IAA influx transporter LAX3 (*Lee et al., 2010*). These results suggest that nematodes hijack IAA distribution network in plants during the colonization of roots in order to facilitate the infection process (*Grunewald et al., 2009a; Haegeman et al., 2012; Lee et al., 2010*). Whereas many studies deal with the suppression of plant defence by fungal and bacterial pathogens, few are dedicated to the suppression of plant defence by nematodes (*Haegeman et al., 2012; Smart and Jones, 2011*).

4.3.2. Protozoa

CK and IAA play an important role in the interaction between *A. thaliana* and the biotrophic protist *Plasmidophora brassicae*, responsible for the club-root disease (formation of aberrant roots) (*Ludwig-Müller and Schuller, 2008*). However, genetic evidence of involvement of CK- and IAA-signalling is sparse. A large screen of mutant lines submitted to *P. brassicae* infection revealed that IAA metabolism, IAA- and ET-signalling mutants are affected by the disease (*Siemens et al., 2002*). However, in another study, *A. thaliana* loss-of-function *axr1-3* mutant appears to be less susceptible than the WT (*Alix et al., 2007*). Moreover, transcriptome analysis of *A. thaliana* infection by *P. brassicae* identified a differential regulation of genes involved in IAA homeostasis and CK metabolism and signalling (*Siemens et al., 2006*).

5. Soil organisms without physical contact with plant roots

Although interactions between plants and soil organisms with a physical contact are the most obvious, other free-living organisms can interact with plants via hormone signalling pathways. Some bacteria and fungus colonize the plant rhizosphere and emit signals at a distance. Recent studies show that other larger organisms belonging to micro-, meso- and macro-fauna living in soil or litter, induce changes in hormone signalling pathways, sometimes through modification in microbial communities and their emitted signals.
5.1. Free living micro-organisms

In contrast with previously described organisms which are closely associated with roots, these micro-organisms generally do not penetrate root tissues. Because such distant interactions have first been observed for micro-organisms with a positive effect on plants, these organisms have been called plant growth promoting bacteria/rhizobacteria or fungus (PGPB/PGPR and PGPF, respectively) (Rashan and Holguin, 1998; Contreras-Cornejo et al., 2009; Kloepper and Schroth, 1978). However, further investigations have demonstrated that some distant interactions are negative for the plant and the category of the Deleterious Rhizo-Bacteria has been proposed (Nehl et al., 1996). The decrease of plant growth by DRB is subtle to identify (absence of necroses), which explains somewhat why they have received less attention. Moreover, the frontier between DRB and PGPR is thin and porous: some PGPR could act as DRB according to plant host genotype, soil environmental factors and time (Nehl et al., 1996). The most widely accepted mechanism for plant growth promotion by PGPR is the production of IAA in the rhizosphere. However, it was also shown that PGPR can produce other plant hormones (Frankenberg and Arshad, 1995) and are able to interact and change plant development in a hormone-signalling dependent or independent way.

5.1.1. Plant growth promoting rhizobacteria

The involvement of IAA-mediated changes in plant development in response to PGPR has been firmly demonstrated (Desbrosses et al., 2012; Persello-Cartieaux et al., 2003). The first study investigating the involvement of IAA signalling in plant response to PGPR showed that the effect of *Pseudomonas thivervalensis* in *A. thaliana* growth was lost in the loss-of-function aux1-100 mutant. In counterpart GA-, ABA-, ET-, JA- and CK-insensitive mutants show the same response as the WT, suggesting that these hormones are not involved in MLG45 plant growth promotion (Persello-Cartieaux et al., 2001). Lateral root growth stimulation by *Phyllobacterium brassicaearum* STM196 is totally abolished in aux1 and axr1 mutants, respectively, impaired in IAA transport and IAA signalling (Contesto et al., 2010). Impact of *Pseudomonas fluorescens* WCS417 in root hair formation was severely affected in loss-of-function tir1arf2arf3 and axr2-1 mutants affected in IAA signalling (Zamioudis et al., 2013).

The effect of free-living bacteria on IAA signalling pathway is not always associated with a production of bacterial IAA: it can be due to other signal molecules interfering with plant IAA signalling pathways. This has been demonstrated with *Bacillus subtilis* GB03 and *P. brassicaearum* STM196 which triggers changes in IAA homeostasis and requires both IAA signalling and transport pathway, independently of any IAA release by the bacteria (Contesto et al., 2010; Zhang et al., 2007). In addition, growth promotion by *Pseudomonas aeruginosa* involves diketopiperazines (DKPs), small molecules synthesized by this bacterium. Modulation of root architecture by DKPs is lost in loss-of-function tir1, arf7-1 and arf9-1 mutants, suggesting again that without any IAA production, the IAA signalling pathway is involved in signal transduction and integration (Ortiz-Castro et al., 2011).

In comparison with IAA-mediated plant growth promotion by PGPR, other hormones have received less attention despite a likely important role. The effect of *Variovorax paradoxus* SC-2 on WT is not retrieved in ET-insensitive loss-of-function etr1-1 and ein2-1 mutants (Chen et al., 2013). Loss of growth promotion by *B. subtilis* GB03 on cre1 mutants suggests that CK signalling plays also an important role in growth promotion by this bacterial strain (Ryu et al., 2003). All combined, these results highlight the involvement of hormone signalling in growth promotion of plants by soil microorganisms. However, adaptive mechanisms involved in plant growth promotion are still elusive. Until recently, major studies on the elicitation of plant growth promotion by PGPR focused on the isolation of bacterial strains according to their ability to produce hormonal compounds or interact directly with plant hormones, sometimes without considering the actual consequences on plant physiology and morphology. For instance, some PGPR carry the enzyme Act5 involved in the degradation of ACC the precursor of ET and thus their growth promoting effects have been attributed to changes in plant ET homeostasis. However, changes in root architecture and growth induced by *P. brassicaearum* STM196, *Pseudomonas putida* UW4, *Rhizobium leguminosarum* 128C53 K and *M. loti* MAFF303099 were conserved in *A. thaliana* loss-of-function mutants impaired in ET-signalling (etr1, ctr1, ein2 and ein3) (Contesto et al., 2008; Galland et al., 2012). These results suggest that ET was finally not involved in the effect of these strains on root architecture and plant growth. In conclusion, the fact that a microorganism is able to produce IAA or enzymes involved in the biosynthesis of other hormones, such as ET, is not a clue that these hormones are necessarily involved in the observed effects on plant (Desbrosses et al., 2012; López-Bucio et al., 2007).

Plant development is not the only aspect of plant growth under the influence of PGPR and DRB: immunity through defence and infection mechanisms can also be modulated. Some of these PGPR confer a systemic broad spectrum resistance against several plant pathogens, making them promising biocontrol agents (Meena, 2012; van Loon et al., 1998). The mechanism under this induced systemic resistance (ISR) is similar to those activated during interaction with pathogens (cf. 2.4 paragraph). It lies upon the detection of microbe-associated molecular patterns (MAMP) of these PGPR by plants. Among these MAMPs, flagellins or lipopolysaccharides (LPS) are the best studied (for review see Bakker et al., 2007). The role played by ET- and JA-signalling in the regulation of *A. thaliana* ISR response is demonstrated for PGPR such as *P. fluorescens* WCS417r (Hase et al., 2003; Pieterse et al., 1998), *P. fluorescens* CHAO (Iavicoli et al., 2003) and *P. putida* LSW175 (Ahn et al., 2007). These PGPR failed to elicit ISR on loss-of-function ET-signalling mutants (etr1-1 and ein) and JA-signalling mutants (jin and jar1) upon *Pseudomonas syringae* pv. *tomato* (Pst) challenge. Moreover, ISR elicited by *B. subtilis* GB03 is dependent on ET-signalling (ein2) but not on JA-signalling (coi1) (Ryu et al., 2004). Finally, in almost all these studies, elicited ISR was lost in the loss-of-function npr1 signalling mutant, with an impaired knob between ISR and SAR, but is generally elicited independently of SA-signalling, demonstrating the importance of NPR1 in the crosstalk between ISR and SAR (Ahn et al., 2007; Iavicoli et al., 2003; Pieterse et al., 1998).

5.1.2. Plant growth promoting fungus

Like growth promotion by free-living bacteria (PGPR), plant growth promotion by free-living fungus (PGPF) requires IAA-signalling. Shoot growth promotion due to the PGPF *Trichoderma virens* is lost in *A. thaliana* aux1-7, ein1-1 IAA-transport mutants and axr1-3 IAA-signalling mutants (Contreras-Cornejo et al., 2009). However, for other PGPF strains like *Aspergillus ustus*, *A. thaliana* growth is still promoted in loss-of-function IAA- (aux1-7 and axr4-1), ET- (etr1-3 and ein1-1), ABA- (abi1-4) and CK- (ahk2-2 and ahk3-2) resistant mutants (Salas-Marina, 2011) showing that hormone-signalling independent mechanisms also exist for PGPF.

As PGPF, PGPR can elicit ISR in an ET- and JA-signalling dependent mechanism. In *A. thaliana*, *P. Indica* (Stein et al., 2008), *Penicillium* sp. GP16-2 (Hossain et al., 2008) and *Trichoderma harzianum* (Korolev et al., 2008) elicited ISR is lost in loss-of-function *A. thaliana* mutants impaired in ET- and JA-signalling. The oomycete *Pythium oligandrum* also elicits ISR in tomato, but this elicitation is lost in loss-of-function jai1-1 mutant (Hase et al., 2008). There is also an antagonism between ISR and SAR elicited by PGPF: ISR is lost in the loss-of-function npr1 signalling mutant...
but is generally elicited independently of SA-signalling (Hase et al., 2008; Hossain et al., 2008; Stein et al., 2008).

### 5.2. Microfauna

Beneficial protozoa are poorly studied. To our knowledge, only one study investigated hormone signalling in the relationship between a beneficial protist and *A. thaliana*. In their study, Krome et al. (2010) using transgenic *DRS5::GUS* IAA marker line and *ARR5::GUS* CK marker line showed that growth promotion of *A. thaliana* by *Acanthamoeba castellanii* is mediated by changes in CK homeostasis but not IAA (Krome et al., 2010).

### 5.3. Mesofauna

All soil organisms with a size between 100 \(\mu\)m and 2 mm belong to the mesofauna (Decaëns, 2010). The main taxa are Acari, Collembola, Diplura, Symphyla, Enchytraeidae, Isotera/Formicoidea and Dipitera. The effect of these organisms on plant growth is almost never studied, except for collembolan. There are few evidences suggesting the involvement of plant hormone signalling in their effect. To our knowledge, the only study suggesting such mechanisms was made by Endlweber et al. (2011). These authors performed a transcriptomic analysis of *A. thaliana* in response to *Protaphorura fimata* showing a transcript accumulation for several IAA-responsive genes but also in defence genes such as *PDF1.2* and members of the *PR* family (Endlweber et al., 2011).

### 5.4. Macrofauna

Soil animals with a size above 2 mm are considered belonging to the macrofauna (Decaëns, 2010). This category comprises Isopoda, Myriapoda, Arachnida, Coleoptera, Mollusca, Oligochaeta (such as earthworms) and Insecta (such as ants and termites) (Decaëns, 2010; Lavelle and Spain, 2001). For a long time, the effect of large soil organisms on plant growth was only studied by soil ecologists. The main mechanisms proposed to explain these effects were the changes in soil physical structure or trophic effect due to the mineralization of soil organic matter which makes nutrients available to the plant (Blouin et al., 2013; Brown et al., 2004). Even if these mechanisms could be of importance on the long term, these hypotheses have been rejected in a series of short term laboratory experiments (Blouin et al., 2006, 2007; Laossi et al., 2010). An experiment on earthworm effect on plant, with earthworm dejections (or "casts") isolated in an *in vitro* device preventing the occurrence of physical or trophic effects, showed that signal molecules diffusing from earthworm casts were sufficient to explain the effect of earthworms on plant growth (Puga-Freitas et al., 2012). Among these diffusible signal molecules, IAA might be the most likely candidate to explain these effects since it has been isolated in earthworm casts and vermicompost (Canelas et al., 2002; Muscolo et al., 1998; Nardi et al., 2000). Humic acids (HA) isolated from vermicompost enhanced enhanced plasma membrane H⁺-ATPase activity in maize roots (Canellas et al., 2011). Moreover, dwarf phenotype of loss-of-function *aux1-7axr4-2* double mutant, known to be rescued by exogenous supply of IAA, is reverted towards WT phenotype in the presence of earthworms (Puga-Freitas et al., 2012). This result was interpreted as a...
first evidence that earthworms modify IAA production, transport or signalling by emitting signal molecules, eventually IAA, in plant rhizosphere.

Along with their impact on plant development, these organisms also interact with plant diseases. Earthworms are considered as a biocontrol agent against several plant pathogens (Fig. 3). For many years, this biocontrol effect was attributed to a direct interaction between earthworms and the pathogen/parasite such as predation, habitat destruction, competition for organic matter, production of fungicides or bactericides decreasing pathogen population (Brown et al., 2004). However, recent studies have demonstrated that earthworm were responsible for the modulation of the expression of PLDα and SOD genes known to be stress responsive (Jana et al., 2010). Reduction of density of the root pathogenic nematode Heterodera sacchari is accompanied by modulation in the expression of stress related genes, not by a decrease in nematode population (Blouin et al., 2005). These first studies were later completed by analyzing A. thaliana transcriptome during the interaction with Aporrectodea caliginosa (Puga-Freitas et al., 2012). Genes known to be stress-inducible and dependent on ET- or JA-signalling are modulated in the presence of earthworms. Several of these genes such as PDF1.2 and PR-4 are known to be modulated during the elicitation of ISR by bacterial elicitors (van Loon et al., 2006).

6. Conclusions and perspectives

Plant hormone signalling pathways are essential for the physiological response of plants to soil organisms. As summarized in Table 1, involvement of the different hormone signalling pathways has been extensively studied for physically associated microorganisms such as Rhizobium, AM and ECM but also for free-living micro-organisms such as PGPR and PGPF. Our knowledge for larger organisms is still sparse. For almost all referenced soil organisms, plant development modification has been shown under the influence of IAA-signalling pathway, excepted for free-living protozoa endophytic fungi. For these two groups, only CK-signalling is documented as playing an essential role in the modification of plant development. For Rhizobium, the most studied soil organism, nodule formation is known to be under the influence of the cross-talk between IAA and CK. ET-signalling seems also a key feature in plant development modifications in response to Agrobacterium, AM, ECM and PGPR. Therefore, physically associated and free-living soil organisms share the same signalling pathways (IAA, CK and ET) in the modification of plant development.

Concerning plant defence mechanisms, interaction between plant and soil organisms involve one or more classical stress related hormone (JA, ET and SA). These hormones are involved in the control of the infection for physically associated organisms or in ISR elicited by free-living organisms. Our survey confirms the antagonism between SAR and ISR by the fact that each category of organism influences either SA-signalling pathway involved in SAR or JA- and ET-signalling pathway involved in ISR, but not both pathways simultaneously (Table 1). Rhizobium appears as an exception to this rule, maybe due to the close physiological relationships with legumes inherited from a strict co-evolutionary process. GA-signalling pathway is involved in plant response to Rhizobium and endophytic fungus. GA–IAA–ABA signalling pathways, well known for their role in plant infection by aboveground organisms, gains importance in plant response to belowground pathogens (Grant and Jones, 2009; Robert-Seilaniantz et al., 2011).

The role of hormonal signalling pathways in ecological interactions between soil organisms and plants is a field of research which takes a growing importance, especially due to the diversity of soil organisms interfering with plants through this common mechanism (Fig. 3). One of the main impediments in this research area is the understanding of hormone signalling pathways and cross-talks between these pathways. Progress in this domain could help in deciphering the links between the presence of one single organism, multiple signal molecule and diverse consequences on plant development and immunity (Hernández-Mata et al., 2010; Ren et al., 2008). Moreover, in the wild, plants interact with a complex community of soil organisms establishing multiple and simultaneous molecular dialogues with this plant (Fig. 3). In this review, plant balance between the different hormones emerges as the hub where multiple signals are integrated to produce a coordinated response and adaptation to biotic environment. Modelling approach should be developed in this sense to identify critical situations where a shift in hormone signalling can affect plant fitness. Huge database providing comprehensive genetic and phenotypic information about hormone-related processes in plants could be helpful (Peng et al., 2009).

To fully understand plant response to its biotic environment, we suggest a systematic analysis of soil microorganism community. Microorganism impact on plant signalling pathways is generally studied with one single isolated strain. However, results obtained in single stress experiments are sometimes very different from results of multi stress experiments (Prasch and Sonnewald, 2013). In parallel of deciphering the impact of one model soil organism on one model plant for one specific pathway, research effort could also be made to consider the multi-factorial environment of plants responsible for their actual adaptations. For example, studying microbial community in extenso, in a natural soil, allows understanding the disease suppressiveness of several plant diseases (Mendes et al., 2011; Sanguin et al., 2009; Weller et al., 2002). The need to characterize the whole microbial community is also required when studying the effect of soil fauna on plants. Indeed, microorganisms are often considered as a black box, whereas they play a central role in microfauna-plant interactions, as demonstrated for the effect of protozoa on plant growth (Bonkowski, 2004; Krome et al., 2010). Soil meso- and macro-fauna influence on plant is also mediated through changes in diversity or abundance of soil microorganisms such as PGPR (Elmer, 2009; Endlweber et al., 2011; Puga-Freitas et al., 2012; Wurst, 2013) (Fig. 3). The study of tripartite interactions between plants, soil fauna and microorganisms requires transdisciplinary approaches linking plant physiology, soil ecology and microbial ecology, by taking advantage of new generation sequencing methods for environmental genomics.

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