Phenolic acids act as signaling molecules in plant-microbe symbioses

Santi M. Mandal,1,2 Dipjyoti Chakraborty3,4 and Satyahari Dey1,*

1Department of Biotechnology; Indian Institute of Technology; Kharagpur, WB India; 2The University of Texas Medical Branch; Galveston, TX USA; 3Plant Molecular & Cellular Genetics; Bose Institute; Kolkata, WB India; 4Department of Bioscience & Biotechnology; Banasthali University; Rajasthan, India

Key words: Agrobacterium sp., flavonoids, legume-rhizobium symbioses, phenolic acids, plant defense, vesicular arbuscular mycorrhiza

Phenolic acids are the main polyphenols made by plants. These compounds have diverse functions and are immensely important in plant-microbe interactions/symbiosis. Phenolic compounds act as signaling molecules in the initiation of legume-rhizobia symbioses, establishment of arbuscular mycorrhizal symbioses and can act as agents in plant defense. Flavonoids are a diverse class of polyphenolic compounds that have received considerable attention as signaling molecules involved in plant-microbe interactions compared to the more widely distributed, simple phenolic acids; hydroxybenzoic and hydroxycinnamic acids, which are both derived from the general phenylpropanoid pathway. This review describes the well-known roles attributed to phenolic compounds as nod gene inducers of legume-rhizobia symbioses, their roles in induction of the GmGin1 gene in fungus for establishment of arbuscular mycorrhizal symbiosis, their roles in inducing vir gene expression in Agrobacterium, and their roles as defense molecules operating against soil-borne pathogens that could have great implications for rhizospheric microbial ecology. Amongst plant phenolics we have a lack of knowledge concerning the roles of phenolic acids as signaling molecules beyond the relatively well-defined roles of flavonoids. This may be addressed through the use of plant mutants defective in phenolic acids biosynthesis or knock down target genes in future investigations.

Introduction

Phenolic acids and their derivatives are a diverse class of phenolic compounds made by plants. They are known to play multifunctional roles in rhizospheric plant-microbe interactions. Phenolic acids are produced in plants via shikimic acid through the phenylpropanoid pathway, as by-products of the monolignol pathway and as breakdown products of lignin and cell wall polymers in vascular plant. Additionally, some phenolic acids are of microbial origin. The phenolic acids found in plant cell walls and lignin have a unique chemical structure of C6-C3 (phenylpropanoid type) whereas those of microbial origin are of the form C6-C1 (Phenylmethyl type).

In response to microbial attack, plants activate defense responses that lead to induction of a broad spectrum of antimicrobial compounds some of which may be species specific. These induced defense mechanisms are expressed at site of attack (hypersensitive response) as well as at a distance (signaled by methyl salicylate) to the site of primary infection and protect the plant from the spread of infection and future attack. Induced resistance is regulated by a network of interconnecting signal transduction pathways in which phenolic acids are key signaling molecules. Phenolic acids are relatively resistant biochemical species; they undergo transformation in the soil because some microorganisms have the capacity to utilize them as carbon sources. It is well established that several soil bacteria have the ability to oxidize aromatic compounds. Simple phenolic compounds, such as methoxy and hydroxy benzoic acid and cinnamic acids, are commonly formed in decaying plant residues. Phenolic acids may provide alternative carbon sources for some diazotrophs in limited environments and may also serve as precursors for the synthesis of phenolic lipids.

Mycorrhizae are formed in symbiotic interactions between plants and fungi. Growth promotion in plants can be attributed to arbuscular mycorrhizal fungal (AMF) associations in some situations such as macronutrient-limited soils, because mycorrhizal plants grow better than non-mycorrhizal plants. Considerable increases in phenolic compounds in host plants as a result of arbuscular mycorrhizal (AM) fungus inoculation have been reported. Generally, inoculation of plants with AMF results in an overall increase in the production of some new phenolic compounds during the progression of the infection.

In legumes, phenolic acids are released rapidly from emerging roots during seed germination and seedling growth. The Rhizobium community in the rhizosphere undergoes changes in response to phenolic acids when they accumulate in the soil which provides a competitive advantage for nodulation by selective rhizobial strains. It is well documented that Rhizobia species have the ability to utilize phenolic acids as a carbon source. A range of soluble and conjugated phenolic acids involved in rhizobial defense and nodule morphogenesis have been detected in roots and root nodules of Arachis hypogaea L. Recently, it has been shown that some endogenous phenolic acids present in root nodules of Vigna mongo stimulate the efficiency of IAA production by its symbionts (Rhizobium sp.) and regulate nodule morphogenesis. Over the last few decades, various functions for these phenolic compounds in root nodules have been investigated.
products’ that bestow metabolic plasticity essential for anticipating and responding to biotic and abiotic stress(es). Such metabolites are generally derived from isopropanoid, phenylpropanoid, alkaloid or fatty acid pathways. All terpenoids are synthesized from the five carbon precursor, isopentenyl diphosphate. Alkaloids are synthesized principally from amino acids and phenolics are derived from either the shikimic acid pathway or the malonate acetate pathway or both. Phenolic acids, characterised by hydroxylated aromatic ring(s) are ubiquitous secondary metabolites in plants and provide one of the most studied and widely exploited metabolic pathways in plant research.

Table 1. Phenolic acids as inducer of plant-microbe symbioses in some selected species

<table>
<thead>
<tr>
<th>Phenolic acids</th>
<th>Function/location</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>caffeic acid</td>
<td>concentration dependent root growth inhibition</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>present in wall bound fraction of nodules</td>
<td>25</td>
</tr>
<tr>
<td>cinnamic acid</td>
<td>induced by Rhizobia in rice and resistant to Rhizoctonia</td>
<td>148</td>
</tr>
<tr>
<td>3,4-dihydroxybenzoic acid</td>
<td>chemo-attractants, influence the host range of the interaction</td>
<td>149</td>
</tr>
<tr>
<td>ferulic acid</td>
<td>concentration dependent root growth inhibition</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>induced by Rhizobia in rice and resistant to Rhizoctonia</td>
<td>148</td>
</tr>
<tr>
<td>gallic acid</td>
<td>induced by Rhizobia in rice and resistant to Rhizoctonia</td>
<td>148</td>
</tr>
<tr>
<td>gallic acid and its methyl ester</td>
<td>reported from soybean nodules—act primarily as antioxidants</td>
<td>150</td>
</tr>
<tr>
<td>p-coumaric acid</td>
<td>Stimulator of IAA production in Rhizobia</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>constitutively present in root and nodule</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Influence rhizobial growth</td>
<td>64</td>
</tr>
<tr>
<td>4-methoxycinnamic acid</td>
<td>phytoanticipant in pea plants</td>
<td>151</td>
</tr>
<tr>
<td>p-hydroxybenzoic acid</td>
<td>chemo-attractants, influence the host range of the interaction</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>Present in soluble fraction of young nodules</td>
<td>25</td>
</tr>
<tr>
<td>4-hydroxybenzaldehyde</td>
<td>Stimulator of IAA production in Rhizobia</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>concentration dependent root growth inhibition</td>
<td>147</td>
</tr>
<tr>
<td>protocatechuic acid</td>
<td>Stimulator of IAA production in Rhizobia</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>present in wall bound fraction of nodules</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Influence rhizobial growth</td>
<td>64</td>
</tr>
<tr>
<td>salicylic acid</td>
<td>Accumulation in alfalfa roots</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Exogenous application inhibit indeterminate nodulation</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>exogenous SA inhibits early nodulation in soybean</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>auto-regulate nodulation at the step of infection thread formation</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Rhizobacteria mediated induced systemic resistance</td>
<td>144</td>
</tr>
<tr>
<td>syringic acid</td>
<td>Reported from soybean nodules</td>
<td>150</td>
</tr>
<tr>
<td>tannic acid</td>
<td>induced by Rhizobia in rice and resistant to Rhizoctonia</td>
<td>148</td>
</tr>
<tr>
<td>vanillic acid</td>
<td>Influence rhizobial growth</td>
<td>64</td>
</tr>
<tr>
<td>vanillin</td>
<td>nod gene inducer in peanut</td>
<td>85</td>
</tr>
<tr>
<td>vanillyl alcohol</td>
<td>chemo-attractants, influence the host range of the interaction</td>
<td>149</td>
</tr>
<tr>
<td>4-O-β-glucosides of p-hydroxybenzoic, protocatechuic and vanillic acids</td>
<td>reported from soybean nodules—act primarily as antioxidants</td>
<td>150</td>
</tr>
<tr>
<td>5-O-β-glucoside of gentisic acid, gallic acid and its methyl ester</td>
<td>reported from soybean nodules—act primarily as antioxidants</td>
<td>150</td>
</tr>
</tbody>
</table>

(Table 1). Microbes and plants have evolved complex signal exchange mechanisms that allow a specific bacterial species to induce its host plant to form invasion structures through which the bacteria can enter the plant root or shoot. This review aims to summarize: how rhizosphere microbial communities impact phenolic signaling and how metabolic plasticity in microsymbionts alters the symbiotic morphogenesis that follows phenolic signaling?

Biosynthesis of Phenolic Acids

Plants produce an extremely diverse array of low molecular mass compounds, often called secondary metabolites or ‘natural products’ that bestow metabolic plasticity essential for anticipating and responding to biotic and abiotic stress(es). Such metabolites are generally derived from isopropanoid, phenylpropanoid, alkaloid or fatty acid pathways. All terpenoids are synthesized from the five carbon precursor, isopentenyl diphosphate. Alkaloids are synthesized principally from amino acids and phenolics are derived from either the shikimic acid pathway or the malonate acetate pathway or both. Phenolic acids, characterised by hydroxylated aromatic ring(s) are ubiquitous secondary metabolites in plants and provide one of the most studied and widely exploited metabolic pathways in plant research.
the presence of PAL homologs in prokaryotes do deserve special mention. The general absence of phenylpropanoids and flavonoids in bacteria was attributed to the absence of the enzymes, PAL and chalone synthase. PAL has been encountered in a few prokaryotes such as the marine bacterium *Streptomyces maritimus*, *Sorangium cellulosum* and *Streptomyces verticillatus*. PAL products are a key component in the benzoyl-coenzyme A pathway in *S. maritimus* for the production of benzoate—primed polyketides enterocin and wailupemycin G. Disruption of the *encP* gene in *S. maritimus* inhibited the production of cinnamate and enterocin. Enterocins are characterised by their broad range of activity against gram positive bacteria and play an important role in maintaining bacterial community structures. PAL proteins have also been reported recently from cyanobacteria that are similar in tertiary and quaternary structure to plant and yeast PALs and associate with secondary metabolite biosynthetic gene clusters as observed for other eubacterial PAL genes. Prokaryotic PALs have been suggested as a viable alternative to plant and yeast PAL in enzyme substitution therapy in patients suffering from phenylketonuria. Evidence for the existence of phenylpropanoid pathway and flavonoid biosynthesis in the industrially important fungus *Aspergillus oryzae* raises new questions about UV light treatment in decontamination of food products as well as opening new avenues for improved production of important and novel metabolites. The superfamily of ammonia lyases also includes histidine ammonia lyase (HAL), common in both prokaryotes and eukaryotes catalyzing deamination of L-His in the histidine degradation pathway and tyrosine ammonia lyase (TAL) that specifically deaminates L-Tyr to p-coumaric acid. Recent research converting simple carbohydrate precursors derived from glycolysis and the pentose phosphate pathway derived to the aromatic amino acids, phenylalanine and tryptophan (Fig. 1). The malonic acid pathway is of less significance in the formation of phenolic acids in higher plants compared to fungi and bacteria. Flavonoids, biosynthetically derived from malonyl CoA and p-coumaroyl CoA, derived from acetate and shikimate respectively, are the largest single group of phenolic C15 compounds composed of two phenolic rings connected by a three carbon unit. They may be considered as phenolic acid derivatives. First described by Koukol and Conn, phenylalanine ammonia lyase (PAL) mediates the formation of cinnamic acid from phenylalanine; is a pivotal branch point of primary and secondary metabolism and is the first and most important regulatory step in the formation of many phenolic acids. The production of phenolics is modulated in response to various stimuli by PAL gene expression and protein activity by mechanisms involving feedback regulation, post transcripational changes and metabolite channeling. It has been shown in transgenic tobacco that the subcellular localization of two PAL isoforms (PAL 1 and PAL 2) is different and depends on the complex between cinnamate 4-hydroxylase (C4H) and PAL 1 which is believed to help partitioning phenylpropanoid biosynthesis into different branch pathways by differential subcellular distribution of cinnamic acid. However in yeast, no interaction was detected in effective channeling of carbon through PAL to p-coumaric acid. The PAL-mediated phenolic pathways of general phenylpropanoid metabolism have been studied extensively and do not fall within the preview of this review. However the recent reports of the presence of PAL homologs in prokaryotes do deserve special mention. The general absence of phenylpropanoids and flavonoids in bacteria was attributed to the absence of the enzymes, PAL and chalone synthase. PAL has been encountered in a few prokaryotes such as the marine bacterium *Streptomyces maritimus*, *Sorangium cellulosum* and *Streptomyces verticillatus*. PAL products are a key component in the benzoyl-coenzyme A pathway in *S. maritimus* for the production of benzoate—primed polyketides enterocin and wailupemycin G. Disruption of the *encP* gene in *S. maritimus* inhibited the production of cinnamate and enterocin. Enterocins are characterized by their broad range of activity against gram positive bacteria and play an important role in maintaining bacterial community structures. PAL proteins have also been reported recently from cyanobacteria that are similar in tertiary and quaternary structure to plant and yeast PALs and associate with secondary metabolite biosynthetic gene clusters as observed for other eubacterial PAL genes. Prokaryotic PALs have been suggested as a viable alternative to plant and yeast PAL in enzyme substitution therapy in patients suffering from phenylketonuria. Evidence for the existence of phenylpropanoid pathway and flavonoid biosynthesis in the industrially important fungus *Aspergillus oryzae* raises new questions about UV light treatment in decontamination of food products as well as opening new avenues for improved production of important and novel metabolites. The superfamily of ammonia lyases also includes histidine ammonia lyase (HAL), common in both prokaryotes and eukaryotes catalyzing deamination of L-His in the histidine degradation pathway and tyrosine ammonia lyase (TAL) that specifically deaminates L-Tyr to p-coumaric acid. Recent research
has extended the understanding of the enzymes of phenylpropanoid metabolism including the upstream enzymes instrumental in recruiting PAL and TAL, a number of reductases, acyltransferases and associated enzymes tailoring phenylpropanoid derived metabolites providing experimental templates for enzyme and metabolite engineering.51

Plant cell walls are composed of polysaccharides contained in the microfibrillar and amorphous phases: cellulose, hemicellulose and pectic substances, as well as the other components: lignin, proteins and enzymes.52 Lignin is crucial for structural integrity of the cell wall and the most abundant biopolymer after cellulose.53 The biosynthesis of lignin involves hydrogenative polymerization of monolignols.54 Monolignol biosynthesis starts with the deamination of phenylalanine and although the pathway is still debated, it involves the phenolic acids p-coumaric, ferulic and sinapic acid and a number of enzymes including caffeoyl-CoA 0-methyltransferase.55 Lignin plays an important role in protecting plants against pathogens. Lignin transformation and decomposition products are generally considered a major source of stable soil organic matter but this process remains poorly understood.56-57 Lignin is considerably resistant to microbial degradation. Some white rot basidiomycetes like *Coriolus versicolor*, *Pleurotus eryngii*, *Phlebia radiata* are typically lignin degrading.58 Complete degradation of lignin model compounds have also been reported by bacteria like *Pseudomonas* sp.59 Lignin degradation by the white rot fungi is a complex process mediated by the action of several extra cellular enzymes, of which lignin peroxidases are the most important.60 Ligninolytic micro-organisms are important in agriculture and industry with a wide tolerance of temperature, pH, oxygen limitation and identification of such organisms from soils by techniques such as DNA fingerprinting is increasingly being applied.61

Phenolic acids are incorporated to cell wall of plants in response to biotic stress with increased flux through the phenylpropanoid pathway resulting in synthesis of cinnamic acid and benzoic acid derivatives that are esterified and incorporated in the cell wall fraction.62 A significant portion of the phenolic acids present in the plants are in conjugated form, principally with a sugar residue linked through one or more of the phenolic hydroxyl groups, or as conjugated esters. Acid or alkali hydrolysis releases a number of soluble phenolic acids that are either associated with lignin or as simple glycosides.63 Phenolics from root and seed exudates, leaf leachates, decaying plant matter play multiple roles in soil formation and pedogenesis influencing mineral elements and organic matter dynamics.64,65 Soil characteristics like pH in turn are important factor in the release of phenolic acid in root exudates. It is reported that at acidic pH, nitrate reduces phenolic exudation and at pH 7.5, it becomes restricted to some root zones.66 Green leaves and decomposing litter can influence rhizosphere nitrogen through phenolics such as chlorogenic acid.67 There is increasing evidence that some plants may directly modify rhizosphere to gain access to unavailable soil nitrogen and phosphorus reserves.68,69 Phenolic exudates are also reported to increase the availability of micro and macronutrients by formation of organic metal complexes.70

**Rhizobium-Legume Symbiosis**

The root nodule is a unique and highly organized structure developed as a result of the symbiotic relationship between leguminous plants and bacteria of the genus *Rhizobium*. Within the root nodule, the invading bacteria (*Rhizobium* sp.) differentiate into nitrogen-fixing bacteroids that provide reduced nitrogen to the plant in exchange for carbohydrates and shelter.71,72 Different aspects of the physiology of legume-Rhizobium symbiosis and mechanism of nitrogen fixation have been extensively investigated by various workers.73,74 The establishment of the symbiosis requires signaling and recognition by both the partners; various signaling molecules are exchanged between the plant and the infecting bacteria to regulate nodule initiation, differentiation and functioning. Thus, many facets of plant-bacterium recognition, nodule formation and nitrogen fixation have been well studied,75,76 but the mechanisms by which root nodules phenylpropanoids regulate the infection efficiency of rhizobia for nodule formation has not been studied in detail. Recent studies in *A. hypogaea* indicate a temporal and spatial differentiation in the accumulation and expression of phenolic acids during nodulation.75

The process of nodule formation in legumes involves the production by the plant of flavonoids, betaines and aldonic acids in its seed and root exudates as signals to the microbial symbiont.77 Although phenolic acids are the main polyphenols involved, their regulation has not received the same attention as flavonoids. Some phenolic compounds from symbiotic legumes are known to promote growth of rhizobial bacteria in the rhizosphere78 and also to serve as chemoattractants that guide rhizobial cells to legume root hairs.79 Fox et al.80 demonstrated that planar phenolic compounds with free hydroxyl groups can interfere with legume-rhizobium flavonoid signalling, and alter the nitrogen fixing symbiosis. Flavonoid production is also induced by rhizobia in roots and nodules81 which regulate *Nod* (nodulation) factor production prior to and during infection.82 It is possible that flavonoids like phenolic acids protect dividing cells from oxidative damage because of their activity as antioxidants.83 Leguminous plants secrete a variety of phenolic compounds from roots such as flavones and flavonols from *Vicia faba*,84 isoflavonoids from soybean,85 and vanillin from grapefruit86 to mention a few. These phenolic compounds regulate *nod* gene expression by the symbiont (*Rhizobium*) and so modify the legume-rhizobial symbiosis. The host root secretes phenolic compounds that act as signaling molecules during expression of various symbiotic plasmid-encoded *nod* (nodulation) genes. Some of these *nod* genes encode enzymes to synthesize a special class of glycolipids (chitooligosaccharides). These signal molecules vary somewhat in structure, but their non-reducing end, which contains an N-acyl long-chain fatty acid, is bioactive in the plant host, triggering root hair deformations and cortical cell divisions within the root leading to nodule formation.

It has been reported that in the co-culture of legumes and *Rhizobium* collectively play a role in the symbiosis.85,86 Some phenolic acids stimulate while others repress *nod* gene expression in *Rhizobium trifolii*.87 Seneviratne and Jayasinghe88
have suggested a possible mechanistic explanation for the effects of phenolic acids on the protein profiles of *Rhizobium*. They showed that the strains are initially induced to produce specific enzymes that are capable of degrading the phenolic acid and interaction of phenolic acids with the rhizobial *nod* gene expression contributes to changes in the protein patterns. The effect of phenolic acids is concentration, and structure dependent, and strain-specific. These potential interactions have implications in modulating nodule formation and in establishing the symbiosis (Table 1).

**The Arbuscular Mycorrhiza Symbiosis**

Arbuscular mycorrhizas (AM) are characterized by the formation of unique arbuscules and vesicles by fungi of the phylum Glomeromycota. Growth promotion is attributed to arbuscular formation of unique arbuscules and vesicles by fungi of the phylum symbiosis. They are present in exudates of plants from diverse taxa to be germination inducers in parasitic plants, have recently been suggested to play a key role in establishment of AM symbiosis. They are present in exudates of plants from diverse taxa and are proposed to be essential signals in establishment of AM association, hyphal branching and directional growth of AMF (arbuscular mycorrhizal fungi) towards root. Strigolactones may provide new avenues in agriculture for the management of parasitic weeds and beneficial fungal symbionts.

**Molecular Response of Micosymbionts to Phenolic Acids**

Phenolic compounds strongly regulate *nod* gene expression of the symbiont (*Rhizobium*) and modify the legume-rhizobial symbiosis. *Rhizobium* releases signal molecules by induction of nodulation (*nod*) genes that control root nodule organogenesis. The expression of these genes is regulated by the *nodD* activator proteins. Rhizobia in turn, respond to the phenolic signal by releasing lipo-chitooligosaccharide *Nod* factors that cause morphological changes in legume root hairs, leading to infection thread formation, nodule development and symbiotic N₂ fixation. Flavonoids are secreted by different root zones and produce divergent effects on *nod*-gene expression and alter nodule organogenesis. The flavonoids released from the plant roots bind to the transcriptional activation sites of *Rhizobial nod* genes (*NodD1/NodD2/NodD3*), and the products of these genes, in turn, activate transcription of other *nod* genes. The *NodD* proteins are localized in the cytoplasmic membrane and interact with flavonoids in the inner membrane. Exchange of a *nodD* gene between *Rhizobium* strains that differ in their sensitivity to different flavonoids changes the sensitivity of the recipient strain to the flavonoid. *Nod* gene protein catalyzes synthesis of different nodulating factors which act to induce the first steps in root nodule initiation.

There is also evidence indicating that flavonoids affect cell division either by regulating auxin transport or its turnover, (thereby regulating auxin accumulation) or by direct involvement in cell cycle regulation. Prinsen et al. have shown that some flavonoids increase IAA production by the symbiont (*Rhizobium sp.*) in vitro and suggested that these flavonoids may exert this
effect through the synthesis of nod signal molecules. Theunis et al.\textsuperscript{117} have demonstrated that flavonoids secreted by host plants, activate expression of the nod gene locus, y\textsuperscript{4}weFG. This gene is located downstream of a nod-box, NB15, and encodes proteins involved in IAA synthesis by Rhizobia, which trigger nodule organogenesis. Flavonoids have also been suggested as potential candidates for endogenous inhibitors of auxin transport in the early stage of nodule initiation.\textsuperscript{118} Studies of auxin import carrier (AUX1) genes in Medicago truncatula indicate that nodule formation requires high auxin levels to initiate the process of cell division and the establishment of the nodule primordium.\textsuperscript{119}

Recently, we observed that some phenolic acids found in root nodules increase the efficiency of IAA production by Rhizobia.\textsuperscript{26} Further studies should therefore be conducted to evaluate the effects of these compounds on symbiosis, nod-gene expression and nodulation efficiency. The bacterium Agrobacterium tumefaciens has been grouped with Rhizobium into the family Rhizobiaceae,\textsuperscript{120} and causes crown gall tumors after infecting the wound sites of mostly dicotyledonous and a few of monocotyledonous plants. A specific segment of the Ti (tumor-inducing) plasmid, the T-DNA, is transferred to host plant cells and is then incorporated into the plant nuclear genome. The virulence (\textit{vir}) genes located on the Ti plasmid, are involved specifically in the processing and transfer of the T-DNA. The \textit{vir} genes are transcriptionally regulated by two members, vir\textit{A} and \textit{virG}.\textsuperscript{121} The \textit{VirA} protein responds to plant signal molecules and then transduces the signal to the \textit{VirG} protein, a response regulator. This protein then binds to upstream regions of each of the \textit{vir} genes and activates transcriptionally \textit{vir} gene expression. \textit{Vir} genes are induced at an acidic pH by phenolic compounds that function in concert with monosaccharides synthesized and exuded from wounded plant cells. Lee et al.\textsuperscript{102,123} clearly stated that the \textit{virA} locus determines which phenolic compounds can function as \textit{vir} gene inducers and the \textit{VirA} protein directly senses the phenolic compounds for \textit{vir} gene activation.

Only a few studies have identified the fungal transcriptional changes induced by plant signals.\textsuperscript{124,125} One important limitation for those studies is low amount of fungal biomass within the root, especially during the colonization phase. Requena et al.\textsuperscript{126} identified few fungal genes regulated during development, used suppressive subtractive hybridization (SSH) to create a subtractive cDNA library from \textit{G. mosseae}. They identified a novel gene (Gm\textit{Gin1}) encoding a two-domain protein that is downregulated in expression upon entry into symbiosis. These authors suggested that Gm\textit{Gin1} could be a sensor for plant signals. This protein is located at the cell membrane, by means of its carboxy terminus, which undergoes splicing in response to signals from the plant. After splicing, the amino terminus remains covalently attached to the plant signal acting as nucleophile. Requena et al.\textsuperscript{127} suggested that a modified \textit{Gin1} is able to exert a signaling function through its ATPase activity and modulate other downstream signaling proteins. They also showed that chemical communication with the plant symbiont modified fungal gene expression as well as induce post-transcriptional modification of fungal proteins to facilitate the development of successful symbioses.

### Phenolic Acid as Defense Molecules

Chester\textsuperscript{28} observed that plants possess defense mechanisms that they use in response to the attack of pathogens. Secondary metabolites play important roles either as local or systemic resistance factors in protecting the plants against various pathogens.\textsuperscript{129-131} Phenolic compounds play a major role in the induction of resistance in plants. Generally, phenolic compounds released from seeds, roots or residue decomposition can act against soil borne pathogens and root-feeding insects.\textsuperscript{132} Roots are a rich source of specific natural products that contribute to the competitiveness of invasive plant species and have a marked effect plant and soilborne organisms.\textsuperscript{133,134} Several studies have shown that plant defense against soil born pathogens, nematodes, phytophagous insects is based on the release and accumulation of phenolic compounds in soil.\textsuperscript{135,136} The activity of particular phenolic compounds depends on their structural diversity. For example, simple and complex phenolic compounds such as cajanin, medicarpin, glyceolin, rotenone, coumestrol, phaseolin, phaseolnin, isoflavonoid, flavonoids act as phytoalexins, phytoanticipins and nematicides against soilborne pathogens and phytophagous insects.\textsuperscript{132,136} Several phenolic acids possess high antifungal activity.\textsuperscript{137,138} Phenolic compounds can offer an alternative to the chemical control of pathogens on agricultural crops.\textsuperscript{136} Accumulation of phenolic compounds at the challenge site also reinforces cell wall which is accompanied by localized production of reactive oxygen species driving cell wall cross linking, antimicrobial activity and defense signaling.\textsuperscript{139} The presence of microorganisms undoubtedly influences the quality and quantity of flavonoids present in the rhizosphere, both through modification of root exudation patterns and microbial catabolism of exudates. Microbial alteration and attenuation of the signals of phenolic compounds may have ecological consequences for plant-microbe interaction.\textsuperscript{140}

Plant phenolic compounds produced during host-pathogen interactions work by several mechanisms in plant defense.\textsuperscript{25,141} It has been suggested that the alteration of flavonoid profiles in response to AMF colonization may be a result of initiation of a general plant defense response which is later suppressed.\textsuperscript{142} Rao and Cooper\textsuperscript{143} have demonstrated that the potential consequences of microbial transformations of pre-existing phenolic compound pools, namely the production of de novo flavonoids which are either nod gene inducers or repressors and also induce rhizobial resistance toward phytoalexins, or the formation of mono-cyclic hydroxy aromatic metabolites which could have implications for competition for nodule occupancy, and chemotactic responses. Such natural defense mechanisms provided by these biomolecules in the rhizosphere deserves more scientific attention because of its dual ecological potential as a sustainable means of reducing soil borne infections and for increasing the soil fertility in the ecosystems. Interestingly, rhizobia induce a number of defense mechanisms in planta thereby conferring increased disease resistance.\textsuperscript{144} Plant growth promoting rhizobacteria (PGPR) are increasingly being used in agriculture with potential beneficial effects on plant growth while limiting deleterious effects of phytopathogens by the production of antimicrobials.\textsuperscript{145} Recent advances in genomic research provide vital clues to the enigma of legume—rhizobia recognition by antimicrobial molecules.\textsuperscript{146}
Future Perspectives and Conclusions

Within the last few decades, strong evidence supporting the roles of phenolic compounds in the establishment of plant-microbe symbioses has been published. Scientists have been focused mostly on the regulation by flavonoid signaling rather than by phenolic acids, although phenolic acids are the major polyphenols in most plants, especially in roots. Obviously there is a complex interrelationship involving phenolic acids and their derivatives such as flavonoids, and the ecology of the plant-microbe symbiosis system. So, microbial attenuation or alteration of phenylpropanoid signals may be an important aspect of rhizosphere ecology and in the establishment of symbiosis. Molecular biological research on plant-microbe symbiosis has addressed several questions about the interaction: (a) how do phenolic acids alter the Rhizobium-legume symbiosis—does this involve binding to transcriptional activation sites (NodD1/NodD2/NodD3) of nod genes, in the same way as it occurs for flavonoids? (Fig. 2) (b) What effects does phenolic acid induction have on the expression (downregulation or upregulation) of nod cluster? (c) Does the catabolism of phenolic acids or their derivatives in the rhizosphere catabolism have effects on the Rhizobium-legume symbiosis? (d) Is there any effect of phenolic acids on nutritional exchange during AM-plant symbiosis? (e) How is the GmGin1 gene in fungus regulated by altering the functional groups of phenolic acids? Regarding the impact of phenolic acids at the plant-microbe symbiosis level, the simplest way to address these questions would be through collective action among researchers to define in detail the genetic, biochemical and physiological parameters of the interactions that involve phenolic acids. Through the use of plant mutants defective in phenolic acid biosynthesis or knock-out mutants of target genes we can also start to address the question of the significance of phenolic acids in plant-microbe symbioses. Major goals in future research will be to identify networks of signals and receptors that provide the gateway to the establishment of plant-microbe symbioses.

Acknowledgements

We greatly acknowledge Prof. Cathe Martin, Department of Metabolic Biology, John Innes Centre, Colney, Norwich, NR4 7UH, United Kingdom, for her valuable insight, necessary correction and advice on this review. We would like to thank Sudipta Mandal Ghosh for references and figures editing in the manuscript.
References


