Chapter 5
Establishment of Actinorhizal Symbioses

Alexandre Tromas, Nathalie Diagne, Issa Diedhiou, Hermann Prodjinoto, Maïmouna Cissoko, Amandine Crabos, Diaga Diouf, Mame Ourèye Sy, Antony Champion, and Laurent Laplaze

5.1 Introduction

To cope with nitrogen limitations, some plants have developed the ability to fix atmospheric nitrogen through symbiotic interaction with soil bacteria. The most efficient and intimate of such associations lead to the formation of new root organs called nodules were bacteria are hosted intracellularly and fix atmospheric nitrogen in optimal conditions. Two types of nitrogen-fixing root nodule symbioses (RNS) have been described: the well-studied legume–rhizobial symbiosis (see Part I) and actinorhizal symbioses (Perrine-Walker et al. 2011). The latter involves filamentous bacteria of the genus *Frankia* that interact with more than 200 plant species from eight different families, collectively called actinorhizal plants (Baker and Mullin 1992). Besides *Datisca* these plants are woody shrubs or trees and are present on every continent except Antarctica (Baker and Schwintzer 1990). The efficiency of nitrogen fixation in actinorhizal symbioses is comparable to the one in

A. Tromas • A. Crabos • A. Champion • L. Laplaze (✉)
Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386 Dakar, Senegal

Institut de Recherche pour le Développement (IRD), UMR DIADE, 911 Avenue Agropolis, 34394 Montpellier cedex 5, France
e-mail: alexandre.tromas@ird.fr; amandine.crabos@ird.fr; antony.champion@ird.fr; laurent.laplaze@ird.fr

N. Diagne • I. Diedhiou • H. Prodjinoto • M. Cissoko
Laboratoire Commun de Microbiologie IRD/ISRA/UCAD, Centre de Recherche de Bel Air, BP 1386 Dakar, Senegal
e-mail: nathalie.diagne@ird.fr; diedhiouissa@yahoo.fr; lheureux018@yahoo.fr; Maimouna.Cissoko@ird.fr

D. Diouf • M.O. Sy
Laboratoire Campus de Biotechnologies Végétales, Département de Biologie Végétale, Faculté des Sciences & Techniques, Université Cheikh Anta Diop, BP 5005 Dakar-Fann, Senegal
e-mail: diaga.diouf@ucad.edu.sn; oureyesy1@yahoo.fr

R. Aroca (ed.), *Symbiotic Endophytes*, Soil Biology 37,
DOI 10.1007/978-3-642-39317-4_5,

legumes with an estimated rate of 240–350 kg ha^{-1} $year^{-1}$ (Wall 2000). Because of this, actinorhizal plants play very important roles in various ecosystems, as pioneer species able to colonise poor or degraded soils and to improve their fertility. These plants are used in soil fixation, agroforestry, reforestation, to build windbreaks or as source of timber or firewood (Diem and Dommergues 1990; Zhong et al. 2011).

Interestingly, phylogenetic analyses have demonstrated that all plant-forming RNS belong to the same clade (Rosid I). It was suggested that this common ancestor possessed some kind of predisposition towards RNS formation (Soltis et al. 1995; Doyle 2011). Studies suggest that actinorhizal symbioses appeared 3–4 times independently during evolution (Swensen 1996).

Despite their ecological importance, the establishment and functioning of actinorhizal symbioses are still poorly understood. Progresses have been hindered by the lack of genetic transformation system in *Frankia* and the fact that actinorhizal plants are mostly trees and shrubs thus making genetic approaches difficult. Moreover, no model system to study actinorhizal symbioses as emerged so far and different groups work with different experimental systems. Nevertheless, technical breakthroughs in the last decade have opened new avenues to the study of actinorhizal symbioses. First of all, the genomes of several *Frankia* strains have been sequenced (Normand et al. 2007; Persson et al. 2011) opening the way for comparative genomic studies (e.g. Bickhart et al. 2009; Udwary et al. 2011) and the analysis of global gene expression during symbiosis (Alloisio et al. 2010). Similarly, genomic resources are now available for some actinorhizal plants including ESTs database and microarrays (Hocher et al. 2006, 2011a). Finally, stable and hairy root transformation has now been achieved for actinorhizal plants of the *Casuarinaceae* (Diouf et al. 1995; Franche et al. 1997), *Datiscaceae* (Markmann et al. 2008) and *Rhamnaceae* (Imanishi et al. 2011) family paving the way for functional plant gene studies through RNAi (Gherbi et al. 2008a, b; Markmann et al. 2008). These new tools have led to advances in our understanding of the molecular mechanisms controlling actinorhizal symbioses formation. In this chapter, we give an overview of the current knowledge on the events leading to actinorhizal symbioses establishment. Multiple reviews addressing specific aspects of actinorhizal symbioses are available for further reading (Péret et al. 2009; Perrine-Walker et al. 2011; Hocher et al. 2011b; Abdel-Lateif et al. 2012; Pawlowski and Demchenko 2012).

5.2 Pre-infection, a Molecular Dialogue Between Symbiotic Partners

The first step towards symbiosis is the recognition of compatible symbionts. Host specificity in actinorhizal symbiosis is not as stringent as in legume–rhizobial symbiosis (Pawlowski and Sprent 2008; Pawlowski and Demchenko 2012). Host specificity originates from both partners. Some *Frankia* subgroups like the

"Casuarina" strains evolved high levels of specificity and are able to nodulate only two *Casuarinaceae* genera, *Casuarina* and *Allocasuarina*. On the plant side, some genera like *Gymnostoma* (*Casuarinaceae*) accept a wide range of *Frankia* strains. In legumes, host specificity derives from co-evolution of the bacterial signal molecule and the plant receptor. For the bacterial signal, specificity would result from chemical substitutions on the same chemical backbone (Wall 2000). We can hypothesise that actinorhizal plants with a broad range of bacterial host would recognise a common feature of the signal molecule whereas actinorhizal plants with restricted bacterial hosts would recognise a specific decoration of the signal molecule. On the *Frankia* side, strains able to nodulate divergent plant species would be able to synthesise multiple molecules corresponding to receptors of various plant species (Pueppke and Broughton 1999).

Nevertheless, little is known about the molecular interactions between *Frankia* and host plants in the rhizosphere prior to infection. It was shown recently that aqueous root exudates from the actinorhizal trees *Casuarina glauca* and *C. cunninghamiana* changed *Frankia* physiology and symbiotic properties (Beauchemin et al. 2012). Root exudates increased the growth of *Frankia* and caused hyphal curling, suggesting a chemotrophic response and/or surface property changes. Interestingly, Beauchemin et al. (2012) showed that root exudates altered the bacterial surface properties at the fatty acid and carbohydrate level. More importantly, *Frankia* cells treated with root exudates formed nodules significantly earlier than controls (Beauchemin et al. 2012). These data support the hypothesis of early chemical signalling between actinorhizal host plants and *Frankia* in the rhizosphere. However, the signals involved have not been identified yet. In legumes, flavonoids have been demonstrated to be the symbiotic plant signal attracting rhizobia and initiating the production of bacterial Nod factors (Ferguson et al. 2010). Flavonoids are secondary metabolites derived from the phenylpropanoid pathway. They are widely distributed in plants and fulfil many functions from pigmentation to cell cycle regulation (Abdel-Lateif et al. 2012; Hassan and Mathesius 2012). The impact of root exudates from *C. cunninghamiana* on *Frankia* could not be mimicked by some flavonoids that were shown to be active in the legume–rhizobia symbiosis (Beauchemin et al. 2012). In *Myrica gale*, flavonoids extracted from fruits changed *Frankia* growth and nitrogen fixation according to the symbiotic specificity of strains, inducing compatible and inhibiting incompatible strains (Popovici et al. 2010). This suggests that flavonoids might be plant signals involved in defining symbiotic specificity. In order to analyse the role of flavonoids in actinorhizal nodule formation, *C. glauca* plants with reduced flavonoids biosynthesis were produced by downregulation of *CgCHS1* (Laplaze et al. 1999) using RNA interference. *CgCHS1* encodes a chalcone synthase, the first enzyme of the flavonoid biosynthetic pathway. In these plants, the level of flavonoids in the roots was drastically reduced (Abdel Lateif and Hocher, in preparation). This led to a delay in nodulation and a reduction of the percentage of nodulation (Abdel Lateif and Hocher, in preparation). These results suggest that flavonoids are important for actinorhizal symbiosis formation and might represent symbiotic signals emitted by the root.

Flavonoids might also be involved in actinorhizal prenodule or nodule development by inhibiting auxin transport as demonstrated in legumes (Hassan and Mathesius 2012).

In the legume–rhizobial symbiosis, flavonoids trigger the expression of the bacterial *nod* genes (Chap. 1). These genes encode proteins involved in the biosynthesis of lipo-chitooligosaccharides molecules named Nod factors that act as bacterial symbiotic signals (Chap. 1). Many aspects of the molecular dialogue between rhizobia and legumes have been elucidated from synthesis to perception and transduction of the symbiotic signals (Chap. 1, Jones et al. 2007). In actinorhizal symbioses, the bacterial signal is not yet identified. The recent sequencing of *Frankia* genomes revealed a lack of canonical *nod* genes essential for Nod factors biosynthesis and that symbiotic genes (such as *nif* genes, *hup1*, *hup2* and *shc*) are not organised in symbiotic island and are not induced under symbiotic conditions in *Frankia* (Alloisio et al. 2010; Normand et al. 2007). Altogether, this suggests that *Frankia* might synthesise chemically distinct signalling molecules. Previous attempts to identify symbiotic signals secreted by *Frankia* led to the isolation of an unknown compound found to be heat stable, hydrophobic, resistant to chitinase and smaller than lipo-chitooligosaccharides (Cérémonie et al. 1999). Besides, *Frankia* is known to produce some auxins such as phenylacetic acid (PAA; Wheeler et al. 1984). Treatment of *Alnus glutinosa* plants with PAA was reported to induce nodule-like structure thus suggesting that these molecules might also be involved in pre-infection signalling (Hammad et al. 2003).

The mechanisms of perception and transduction of the bacterial symbiotic signal in actinorhizal plants are poorly known. Genetic studies in model legumes have revealed that legume-rhizobial and the more ancient arbuscular mycorhizal (AM) symbioses (Part III) share part of their genetic programme leading to endosymbiosis including part of the symbiotic signal transduction pathway. This is in accordance with studies that suggest that the legume–rhizobial symbiosis would be derived from the more ancient AM symbiosis (Parniske 2008). Homologues of the genes involved in the common symbiotic transduction pathway have been identified in EST databases from the actinorhizal plants *C. glauca* and *A. glutinosa* (Hocher et al. 2011a, b). Transcriptome analyses showed that some of these genes are more expressed in actinorhizal nodules compared to non-inoculated roots (Hocher et al. 2011a). The receptor kinase SYMRK is part of the common Nod and Myc signalling pathway. A homologue was recently isolated from two actinorhizal species *C. glauca* and *Datisca glomerata* (Gherbi et al. 2008a; Markmann et al. 2008). Knockdown of *SYMRK* by RNA interference led to inhibition of nodulation and mycorrhisation in these plants when inoculated with compatible *Frankia* bacteria and AM fungi, respectively. Moreover, *C. glauca SYMRK* complemented the *Lotus japonicus ljsymrk* mutant for both nodulation and mycorrhisation (Gherbi et al. 2008a; Markmann et al. 2008). This demonstrated that *SYMRK* is a common signalling element shared between AM, legume–rhizobia and actinorhizal symbioses, supporting the hypothesis that the capacity to accommodate N_2-fixing bacteria evolved at least partly from the more ancient AM genetic programme. Interestingly, complementation of *ljsymrk* using *SYMRK* genes

isolated from nodulating and non-nodulating species showed that all the genes tested were able to complement the lack of mycorrhisation but only genes with three LRRs (found in *Tropaeolum majus* and all nodulating plants) were able to complement the nodulation defect (Markmann et al. 2008). The appearance of an additional LRR motif in SYMRK might therefore be one of the evolutionary events that led to nitrogen-fixing RNS apparition in the Fabid clade.

In conclusion, while chemical signalling between *Frankia* and its host plants seems to involve molecules different from Nod factors, the same common symbiotic transduction pathway was recycled from the more ancient AM symbiosis to perceive symbiotic signals in legume–rhizobial and actinorhizal symbioses.

5.3 Infection

After pre-infection events, *Frankia* can enter the plant root either intracellularly via root hairs or intercellularly via the middle lamellas of cell epidermal (Obertello et al. 2003; Wall and Berry 2008). The same *Frankia* strains can induce both types of infection in different host plants indicating that the type of infection is controlled by the host plant (Racette and Torrey 1989; Pawlowski and Demchenko 2012).

5.3.1 Intracellular Infection

Intracellular infection is found in the *Myrica*, *Comptonia*, *Alnus* and *Casuarina* genera (Berry and Sunnel 1990). *Frankia* bacteria secrete factors that induce root hair deformation (Fig. 5.1a; Callaham and Torrey 1977). Studies carried out by Torrey (1976) demonstrate that all root hairs are deformed during *Casuarina* infection while just some root hairs are deformed in *Comptonia* (Callaham et al. 1979). Sugar-binding lectins produced by *Frankia* might help the bacteria to bind the root hairs in some actinorhizal plants such as *A. glutinosa* (Pujic et al. 2012). *Frankia* hyphae become entrapped by plant cell polysaccharides at the tip of some deformed root hairs and a local hydrolysis of primary cell occurs at the site of *Frankia* penetration (Berry et al. 1986). Surprisingly, recent analyses of *Frankia* genomes did not find any conserved secreted polysaccharide-degrading enzymes that might be responsible for this degradation (Mastronunzio et al. 2008). This might suggest that *Frankia* secretes effector-like molecules to communicate with its host and trigger the local loosening to the cell wall necessary for infection to occur (Mastronunzio et al. 2008). In some deformed root hair, the plasma membrane invaginates and forms an infection thread structure (Fig. 5.1b). Within this structure, growing *Frankia* hyphae are encapsulated by a cell wall-like matrix made of xylan, hemicellulose, cellulose and pectin (Berg 1990).

Root hair deformation occurs 24–28 h after inoculation. However, only growing root hairs are infected by *Frankia* (Callaham et al. 1979). In these infected root

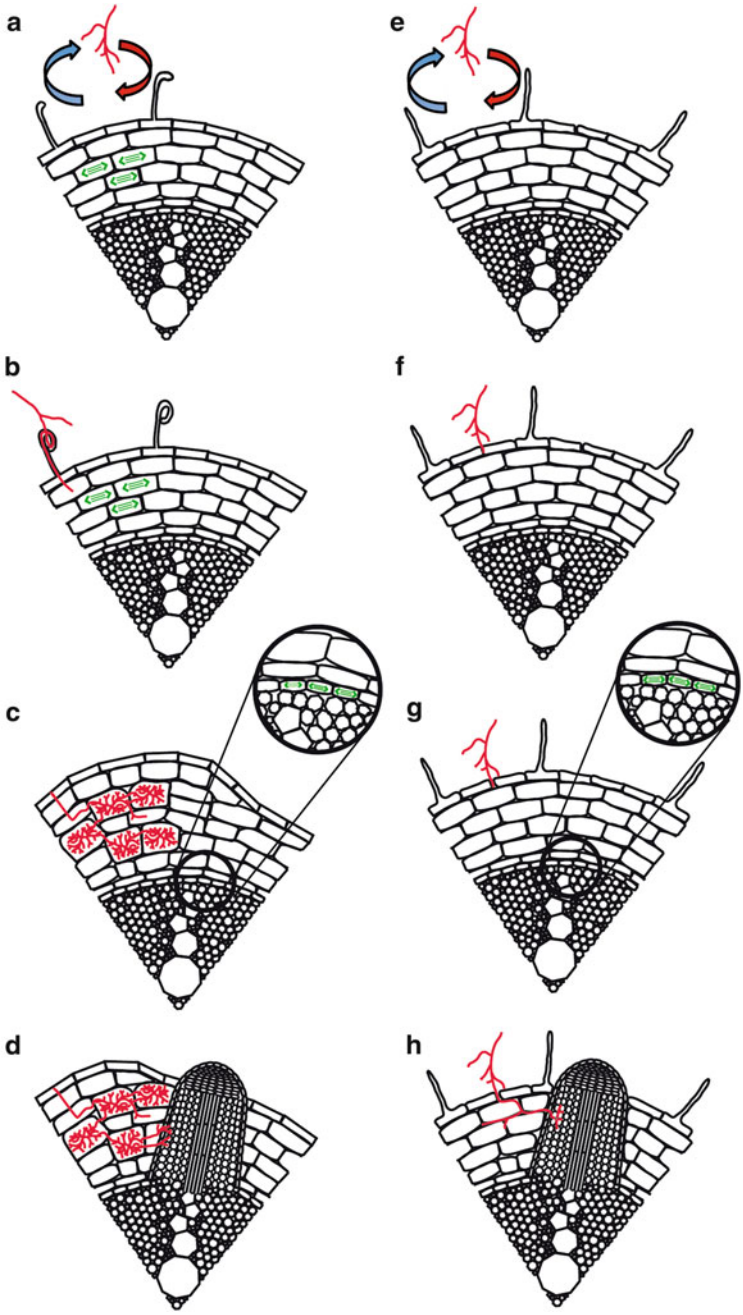

Fig. 5.1 Establishment of actinorhizal symbiosis through intracellular (**a**–**d**) and intercellular infection (**e**–**h**). *Frankia* hyphae are shown in *red* and dividing cells in *green*. (**a**, **e**). Exchange of symbiotic signals between the two partners with, only in (**a**), deformation of root hairs and metabolic modifications in cortical cells close to the site of perception of the bacterial signal. (**b**) Penetration of *Frankia* within a curled root hair and initiation of division in cortical cells.

hairs, a high metabolic activity is observed (Berry et al. 1986; Berry and Sunnel 1990). Simultaneously with infection, cell divisions occur in cortical cells adjacent to the infected root hair inducing the formation of a protuberance called the prenodule (Fig. 5.1b). Infected threads grow towards the prenodule and invade some of its cells that become hypertrophied and both the plant cell and bacteria differentiate to fix nitrogen (Fig. 5.1c; Laplaze et al. 2000a). While the prenodule is an obligatory step of the infection process, it is not the precursor of a nodule lobe. As the prenodule develops, cell divisions occur in the pericycle opposite to a protoxylem pole giving rise to a nodule primordium (Fig. 5.1c). The nodule primordium develops into a nodule lobe that is infected by *Frankia* hyphae coming from the prenodule (Fig. 5.1d).

A gene encoding a protease of the subtilase family called *Cg12* or *Ag12* in *C. glauca* and *A. glutinosa,* respectively, is specifically expressed in plant cells infected by *Frankia* (Laplaze et al. 2000b; Ribeiro et al. 1995; Svistoonoff et al. 2003). No expression was found during intracellular symbiosis with the AM fungus *Glomus intraradices* (Svistoonoff et al. 2003). It has been proposed that these proteases might be involved in cell wall remodelling or the processing of peptidic signals during symbiotic infection. A recent comparative transcriptome analysis of genes induced during actinorhizal, rhizobial and AM symbioses indicates that protease-encoding genes are among the core genes that are induced in all three endosymbioses (Tromas et al. 2012). This suggests that proteases are important component for setting endosymbioses and that some of the genes encoding proteases involved in the ancient AM symbiosis have been recycled to form RNS. Interestingly, the infection-specific induction of a *ProCG12::GUS* construct was retained in the model legume *M. truncatula* (Svistoonoff et al. 2004) indicating that gene regulation during infection in legume–rhizobial and actinorhizal symbioses might use conserved regulators.

Several studies suggest a role of the phytohormone auxin during infection by *Frankia*. A gene named *CgAUX1* encoding a functional auxin influx carrier is expressed in plant cells infected by *Frankia* but not by the AM fungi *G. intraradices* in *C. glauca* (Péret et al. 2007, 2008). Inhibition of auxin influx using 1-naphtoxy acetic acid (1-NOA) delays actinorhizal nodule formation and leads to the formation of small nodules in *C. glauca* (Péret et al. 2007). *Frankia* produces auxins, indole-3-acetic acid (IAA) and phenylacetic acid (PAA), in vitro (Wheeler and Henson 1979; Hammad et al. 2003; Perrine-Walker et al. 2010). Recent immunolocalisation experiments showed specific accumulation of both IAA and PAA in plant cells infected by *Frankia* in *C. glauca* (Perrine-Walker et al. 2010). Gene expression, immunolocalisation and modelling experiments suggest that this specific accumulation is due to auxin production by *Frankia in planta*

Fig. 5.1 (continued) (**f**) Penetration of *Frankia* in between epidermal cells. (**c**) Branching of *Frankia* hyphae within the prenodule and divisions of pericyle cells at the site of initiation of the nodule, in front of a xylem pole. (**g**) Initiation of the nodule without formation of a prenodule. (**d, h**) emergence of the nodule colonised by *Frankia*

and the specific localisation of auxin influx and efflux carriers in *C. glauca* nodules (Perrine-Walker et al. 2010). Altogether, these studies link symbiotic infection to auxin accumulation in *C. glauca*. However, we do not know if this is a common feature of actinorhizal symbioses. Moreover, the role, if any, of auxin in those cells infected by *Frankia* is still unknown. We are currently addressing this question by inhibiting auxin signalling specifically during *Frankia* infection in *C. glauca* (Laplaze, unpublished data).

5.3.2 Intercellular Infection

Intercellular infection occurs in some actinorhizal plant genera such as *Elæagnus*, *Ceanothus*, *Cercocarpus*, *Hippophae*, *Shepherdia* and *Discaria* (Miller and Baker 1985; Berry and Sunnel 1990; Valverde and Wall 1999; Imanishi et al. 2011). During intercellular infection, some signal exchange must occur between the two partners but no root hair deformation is observed (Fig. 5.1e). Instead, *Frankia* enters through the middle lamella between adjacent epidermal cells (Fig. 5.1f) and then progresses intercellularly in the root cortex (Fig. 5.1g). As in intracellular infection, this is associated with pectolytic activity that might be of plant rather than bacterial origin (Mastronunzio et al. 2008). During intercellular infection, prenodule formation has not been reported. However, some cortical cell divisions occur in *Ceanothus* but these new cells are not infected by *Frankia* (Berry and Sunnel 1990). Nodule primordium formation occurs through cell divisions in the pericycle in front of a xylem pole. The nodule lobe primordium is then colonised by intercellular hyphae (Fig. 5.1g). *Frankia* hyphae become intracellular when they invade cortical cells of the young nodule primordium.

5.4 Actinorhizal Nodule Formation

Actinorhizal nodule lobes are formed from cell divisions occurring in the pericycle in front of a xylem pole. New nodule lobes are formed by branching, giving rise to a coralloid actinorhizal nodule formed of multiple lobes. Each lobe contains a meristem at its apex, a central vascular bundle and a periderm. In the nodule, four zones have been defined: (1) the meristematic zone, (2) the infection zone, (3) the fixation zone and (4) the senescence zone. The *meristematic zone* is localised at the apex and produces new cells responsible for the indeterminate growth of actinorhizal nodules. The *infected zone* is adjacent to the apical zone. In this zone, *Frankia* hyphae infect some of the new cells. The *fixation zone* is composed of infected and uninfected cells. Infected cells are filled with *Frankia* hyphae and hypertrophied. Vesicles differentiate and nitrogen fixation occurs. Assimilation of the fixed N probably occurs in uninfected cells (Wall 2000). The *senescence zone* is

localised at the base of old nodules. In this zone, the host cytoplasm and the bacteria degenerate.

Because of its origin, i.e. cells divisions in the pericycle in front of xylem poles and its structure, the actinorhizal nodule lobe has been considered as a modified lateral root (Pawlowski and Bisseling 1996). Moreover, in some actinorhizal plants such as *C. glauca*, a structure called nodular root is formed. The nodular root is a very specialised root showing negative geotropism (growing upward) and cortical aerenchyma and lacks a root cap and root hairs. It has been suggested that it plays an important role under flooding or waterlogged conditions by increasing gas exchange between the nodule and the atmosphere (Silvester et al. 1990). Furthermore, study carried out by Schwintzer et al. (1982) shows a correlation between the external oxygen tension and the length of the modified root. The formation of lenticels is noted at the nodule periderm of certain actinorhizal genera (*Alnus*, *Coriaria* and *Datisca*). This structure is also involved in nodule aeration (Silvester et al. 1990). The presence of both structures, lenticel and root nodule in some Datisca species, has been reported by Pawlowski et al. (2007).

5.5 Conclusions

Nitrogen availability is one of the major limiting factors of crop production worldwide. The high price of nitrogen fertilisers and their environmental impact has recently renewed the interest of the plant science community and funding charities for research on the transfer of biological nitrogen fixation to crops such as cereals (Den Herder et al. 2010; Beatty and Good 2011). Two groups of plants evolved the ability to form nitrogen-fixing RNS: the rhizobial symbiosis is restricted to the *Fabaceae* with the notable exception of the genus *Parasponia* (*Cannabaceae* family), while actinorhizal symbioses occur in eight angiosperm families. Interestingly, molecular phylogenies studies showed that (1) all these plants belong to a single N_2-fixing clade (Soltis et al. 1995) and (2) that nodulation appeared several times independently in both groups (Doyle 2011). This led to the suggestion that the common ancestor of the N_2-fixing clade had a yet unknown genetic predisposition to form RNS. Recent results have shown that actinorhizal and rhizobial symbioses rely on similar molecular components that were recycled from the ancient and widespread AM symbiosis (reviewed in Geurts et al. 2012). More studies on actinorhizal and rhizobial symbioses (including the atypical *Parasponia–Rhizobium* symbiosis) are needed to understand how during evolution RNS appeared in the nitrogen-fixing clade. Moreover, because actinorhizal (and *Parasponia*) nodules are simpler structure (modified lateral roots) that appeared independently in different plant families, it might represent a better model for the transfer of nodulation to other plants. New strategies are being developed to characterise the molecular mechanisms of actinorhizal symbioses formation and functioning. This should shed a new light on the evolution of root endosymbioses and hopefully pave the way to the transfer of nitrogen fixation to important crops.

References

Abdel-Lateif K, Bogusz D, Hocher V (2012) The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and *Frankia* bacteria. Plant Signal Behav 7(6):636–641

Alloisio N, Queiroux C, Fournier P, Pujic P, Normand P, Vallenet D, Médigue C, Yamaura M, Kakoi K, Kucho K (2010) The *Frankia alni* symbiotic transcriptome. Mol Plant Microbe Interact 23:593–607

Baker DD, Mullin BC (1992) Actinorhizal symbioses. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman and Hall, New York, NY, pp 259–292

Baker DD, Schwintzer CR (1990) Introduction. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and Actinorhizal plants. Academic, New York, NY, pp 157–176

Beatty PH, Good AG (2011) Plant science. Future prospects for cereals that fix nitrogen. Science 333:416–417

Beauchemin NJ, Furnholm T, Lavenus J, Svistoonoff S, Doumas P, Bogusz D, Laplaze L, Tisa LS (2012) Casuarina root exudates alter the physiology, surface properties, and plant infectivity of *Frankia* sp. strain CcI3. Appl Environ Microbiol 78:575–580

Berg RH (1990) Cellulose and xylans in the interface capsule in symbiotic cells of actinorhizae. Protoplasma 159:35–43

Berry AL, Sunnel LA (1990) The infection process and nodule development. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, New York, NY, pp 61–81

Berry AM, McIntyre L, McCully M (1986) Fine structure of root hair infection leading to nodulation in the *Frankia-Alnus* symbiosis. Can J Bot 64:292–305

Bickhart DM, Gogarten JP, Lapierre P, Tisa LS, Normand P, Benson DR (2009) Insertion sequence content reflects genome plasticity in strains of the root nodule actinobacterium *Frankia*. BMC Genomics 10:468

Callaham D, Torrey JG (1977) Prenodule formation and primary nodule development in roots of *Comptonia* (Myricaceae). Can J Bot 55:2306–2318

Callaham D, Newcomb W, Torrey JG, Peterson RL (1979) Root hair infection in actinomycete-induced root nodule initiation in *Casuarina*, *Myrica* and *Comptonia*. Bot Gaz 140:S1–S9

Cérémonie H, Debellé F, Fernandez MP (1999) Structural and functional comparison of *Frankia* root hair deforming factor and rhizobia Nod factor. Can J Bot 77:1293–1301

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600–607

Diem HG, Dommergues YR (1990) Current and potential uses and management of Casuarinaceae in the tropics and subtropics. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, CA, pp 317–342

Diouf D, Gherbi H, Prin Y, Franche C, Duhoux E, Bogusz D (1995) Hairy root nodulation of *Casuarina glauca*: a system for the study of symbiotic gene expression in an actinorhizal tree. Mol Plant Microbe Interact 8:532–537

Doyle JJ (2011) Phylogenetic perspectives on the origins of nodulation. Mol Plant Microbe Interact 24:1289–1295

Ferguson BJ, Indrasumunar A, Hayashi S, Lin M-H, Lin Y-H, Reid DE, Gresshoff PM (2010) Molecular analysis of legume nodule development and autoregulation. J Integr Plant Biol 52: 61–76

Franche C, Diouf D, Le QV, Bogusz D, N'Diaye A, Gherbi H, Gobé C, Duhoux E (1997) Genetic transformation of the actinorhizal tree *Allocasuarina verticillata* by *Agrobacterium tumefaciens*. Plant J 11:897–904

Geurts R, Lillo A, Bisseling T (2012) Exploiting an ancient signalling machinery to enjoy a nitrogen fixing symbiosis. Curr Opin Plant Biol 15:1–6

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Péret B, Laplaze L, Franche C et al (2008a) SymRK defines a common genetic basis for plant root

endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and *Frankia* bacteria. Proc Natl Acad Sci 105:4928–4932

Gherbi H, Nambiar-Veetil M, Chonglu Z, Félix J, Autran D, Girardin R, Vaissayre V, Auguy F, Bogusz D, Franche C (2008b) Post-transcriptional gene silencing in the root system of the actinorhizal tree *Allocasuarina verticillata*. Mol Plant Microbe Interact 21:518–524

Hammad Y, Nalin R, Marechal J, Fiasson K, Pepin R, Berry AM, Normand P, Domenach A-M (2003) A possible role for phenyl acetic acid (PAA) on *Alnus glutinosa* nodulation by *Frankia*. Plant Soil 254:193–205

Hassan S, Mathesius U (2012) The role of flavonoids in root–rhizosphere signalling: opportunities and challenges for improving plant–microbe interactions. J Exp Bot 63(9):3429–3444

Hocher V, Auguy F, Argout X, Laplaze L, Franche C, Bogusz D (2006) Expressed sequence-tag analysis in *Casuarina glauca* actinorhizal nodule and root. New Phytol 169:681–688

Hocher V, Alloisio N, Florence A, Fournier P, Doumas P, Pujic P, Gherbi H, Queiroux C, Da Silva C, Wincker P et al (2011a) Transcriptomics of actinorhizal symbioses reveals homologs of the whole common symbiotic signaling cascade. Plant Physiol 156:700–711

Hocher V, Alloisio N, Bogusz D, Normand P (2011b) Early signaling in actinorhizal symbioses. Plant Signal Behav 6:1377–1379

Imanishi L, Vayssières A, Franche C, Bogusz D, Wall L, Svistoonoff S (2011) Transformed hairy roots of the actinorhizal shrub *Discaria trinervis*: a valuable tool for studying actinorhizal symbiosis in the context of intercellular infection. BMC Proc 5:P85

Jones KM, Kobayashi H, Davies BW, Taga ME, Walker GC (2007) How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. Nat Rev Microbiol 5:619–633

Laplaze L, Gherbi H, Frutz T, Pawlowski K, Franche C, Macheix JJ, Auguy F, Bogusz D, Duhoux E (1999) Flavan-containing cells delimit *Frankia*-infected compartments in *Casuarina glauca* nodules. Plant Physiol 121:113–122

Laplaze L, Duhoux E, Franche C, Frutz T, Svistoonoff S, Bisseling T, Bogusz D, Pawlowski K (2000a) *Casuarina glauca* prenodule cells display the same differentiation as the corresponding nodule cells. Mol Plant Microbe Interact 13:107–112

Laplaze L, Ribeiro A, Franche C, Duhoux E, Auguy F, Bogusz D, Pawlowski K (2000b) Characterization of a *Casuarina glauca* nodule-specific subtilisin-like protease gene, a homolog of *Alnus glutinosa* ag12. Mol Plant Microbe Interact 13:113–117

Markmann K, Gábor G, Parniske M (2008) Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6:e68

Mastronunzio JE, Tisa LS, Normand P, Benson DR (2008) Comparative secretome analysis suggests low plant cell wall degrading capacity in *Frankia* symbionts. BMC Genomics 9:47

Miller IM, Baker DD (1985) The initiation, development and structure of root nodules in *Elaeagnus angustifolia* L. (Elaeagnaceae). Protoplasma 128:107–119

Normand P, Lapierre P, Tisa LS, Gogarten Johann P, Alloisio N, Bagnarol E, Bassi CA, Berry AM, Bickhart DM, Choisne N et al (2007) Genome characteristics of facultatively symbiotic *Frankia* sp. strains reflect host range and host plant biogeography. Genome Res 17:7–15

Obertello M, Sy MO, Laplaze L et al (2003) Actinorhizal nitrogen fixing nodules: infection process, molecular biology and genomics. Afr J Biotechnol 2:528–538

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Pawlowski K, Bisseling T (1996) Rhizobial and actinorhizal symbioses: what are the shared features? Plant Cell 8:1899

Pawlowski K, Demchenko KN (2012) The diversity of actinorhizal symbiosis. Protoplasma 249(4): 967–979

Pawlowski K, Sprent JI (2008) Comparison between actinorhizal and legume symbiosis. In: Pawlowski K, Sprent JI, Pawlowski K, Newton WE, Dilworth MJ, James EK, Sprent Janet I, Newton WE (eds) Nitrogen fixation: origins, applications, and research progress, Nitrogen-fixing actinorhizal symbioses. Springer, Dordrecht, pp 261–288

Pawlowski K, Jacobsen KR, Alloisio N, Ford Denison R, Klein M, Tjepkema JD, Winzer T, Sirrenberg A, Guan C, Berry AM (2007) Truncated hemoglobins in actinorhizal nodules of *Datisca glomerata*. Plant Biol (Stuttg) 9:776–785

Péret B, Swarup R, Jansen L, Devos G, Auguy F, Collin M, Santi C, Hocher V, Franche C, Bogusz D et al (2007) Auxin influx activity is associated with *Frankia* infection during actinorhizal nodule formation in *Casuarina glauca*. Plant Physiol 144:1852–1862

Péret B, Svistoonoff S, Lahouze B, Auguy F, Santi C, Doumas P, Laplaze L (2008) A role for auxin during actinorhizal symbioses formation? Plant Signal Behav 3:34–35

Péret B, Svistoonoff S, Laplaze L (2009) When plants socialize: symbioses and root development. In: Beeckman T (ed) Root development, vol 37, Annual plant reviews. Wiley-Blackwell, Hoboken, NJ, pp 209–238

Perrine-Walker F, Doumas P, Lucas M, Vaissayre V, Beauchemin NJ, Band LR, Chopard J, Crabos A, Conejero G, Péret B et al (2010) Auxin carriers localization drives auxin accumulation in plant cells infected by *Frankia* in *Casuarina glauca* actinorhizal nodules. Plant Physiol 154:1372–1380

Perrine-Walker F, Gherbi H, Imanishi L, Hocher V, Ghodhbane-Gtari F, Lavenus J, Benabdoun FM, Nambiar-Veeti M, Svistoonoff S, Laplaze L (2011) Symbiotic signaling in actinorhizal symbioses. Curr Protein Pept Sci 12:156–164

Persson T, Benson DR, Normand P, Vanden Heuvel B, Pujic P, Chertkov O, Teshima H, Bruce DC, Detter C, Tapia R et al (2011) Genome sequence of 'Candidatus Frankia datiscae' Dg1, the uncultured microsymbiont from nitrogen-fixing root nodules of the Dicot *Datisca glomerata*. J Bacteriol 193:7017–7018

Pujic P, Fournier P, Alloisio N, Hay A-E, Maréchal J, Anchisi S, Normand P (2012) Lectin genes in the *Frankia* alni genome. Arch Microbiol 194:47–56

Popovici J, Comte G, Bagnarol E, Alloisio N, Fournier P, Bellvert F, Bertrand C, Fernandez MP (2010) Differential effects of rare specific flavonoids on compatible and incompatible strains in the *Myrica gale-Frankia* actinorhizal symbiosis. Appl Environ Microbiol 76(8):2451–2460

Pueppke SG, Broughton WJ (1999) *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. Mol Plant Microbe Interact 12:293–318

Racette S, Torrey JG (1989) Root nodule initiation in *Gymnostoma* (Casuarinaceae) and *Shepherdia* (Elaeagnaceae) induced by *Frankia* strain HFPGpI1. Can J Bot 67:2873–2879

Ribeiro A, Akkermans AD, van Kammen A, Bisseling T, Pawlowski K (1995) A nodule-specific gene encoding a subtilisin-like protease is expressed in early stages of actinorhizal nodule development. Plant Cell 7:785–794

Schwintzer CR, Berry AM, Disney LD (1982) Seasonal patterns of root nodule growth, endophyte morphology, nitrogenase activity, and shoot development in *Myrica-Gale*. Can J Bot 60: 746–757

Silvester WB, Harris SL, Tjepkema JD (1990) Oxygen regulation and hemoglobin. In: Schwintzer CR, Tjepkema JD (eds) The biology of *Frankia* and actinorhizal plants. Academic, San Diego, CA, pp 157–176

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG (1995) Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. Proc Natl Acad Sci USA 92:2647–2651

Svistoonoff S, Laplaze L, Auguy F, Runions J, Duponnois R, Haseloff J, Franche C, Bogusz D (2003) cg12 expression is specifically linked to infection of root hairs and cortical cells during *Casuarina glauca* and *Allocasuarina verticillata* actinorhizal nodule development. Mol Plant Microbe Interact 16:600–607

Svistoonoff S, Laplaze L, Liang J, Ribeiro A, Gouveia MC, Auguy F, Fevereiro P, Franche C, Bogusz D (2004) Infection-related activation of the cg12 promoter is conserved between actinorhizal and legume-rhizobia root nodule symbiosis. Plant Physiol 136:3191–3197

Swensen SM (1996) The evolution of actinorhizal symbioses: evidence for multiple origins of the symbiotic association. Am J Bot 83:1503–1512

Torrey JG (1976) Initiation and development of root nodules of *Casuarina* (*Casuarinaceae*). Am J Bot 63:335–345

Tromas A, Parizot B, Diagne N, Champion A, Hocher V, Cissoko M, Crabos A, Prodjinoto H, Lahouze B, Bogusz D, Laplaze L, Svistoonoff S (2012) Heart of endosymbioses: transcriptomics reveals a conserved genetic program among arbuscular mycorrhizal, actinorhizal and legume-rhizobial symbioses. PLoS One 7:e44742

Udwary DW, Gontang EA, Jones AC, Jones CS, Schultz AW, Winter JM, Yang JY, Beauchemin N, Capson TL, Clark BR et al (2011) Comparative genomic and proteomic analysis of the actinorhizal symbiont *Frankia* reveals significant natural product biosynthetic potential. Appl Environ Microbiol 77(110):3617–3625

Valverde C, Wall LG (1999) Time course of nodule development in the *Discaria trinervis* (Rhamnaceae)—*Frankia* symbiosis. New Phytol 141:345–354

Wall LG (2000) The actinorhizal symbiosis. J Plant Growth Regul 19:167–182

Wall LG, Berry AM (2008) Early interactions, infection and nodulation in actinorhizal symbiosis. In: Pawlowski K, Newton WE, Dilworth MJ, James EK, Sprent Janet I, Newton WE (eds) Nitrogen fixation: origins, applications, and research progress, Nitrogen-fixing actinorhizal symbioses. Springer, Dordrecht, pp 147–166

Wheeler CT, Henson IE (1979) Hormones in plants bearing actinomycete nodules. Bot Gaz 140: 52–57

Wheeler C, Crozier A, Sandberg G (1984) The biosynthesis of indole-3-acetic acid by *Frankia*. Plant Soil 78:99–104

Zhong C, Pinyopusarek K, Kalinganire A, Franche C (2011) Improving smallholder livelihoods through improved casuarina productivity: proceedings of the 4th international casuarina workshop. China Forestry Publishing House, Haikou