How the Environment Regulates Root Architecture in Dicots

MARIANA JOVANOVIC,* VALÉRIE LEFEBVRE,*,† PHILIPPE LAPORTE,* SILVINA GONZALEZ-RIZZO,* CHRISTINE LELANDAIS-BRIÈRE,*,† FLORIAN FRUGIER,* CAROLINE HARTMANN*,† AND MARTIN CRESPI*

**Institut des Sciences du Végétal, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette, France*
†*Université Paris VII-Denis Diderot, 2 place Jussieu, 75251 Paris Cedex 5, France*

Advances in Botanical Research, Vol. 46
Incorporating Advances in Plant Pathology

0065-2296/08 $35.00
DOI: 10.1016/S0065-2296(07)46002-5

ABSTRACT

The efficient acquisition of soil resources (nutrients and water) through the root system is crucial for crop productivity. In order to adapt root growth to the soil environment, plants can optimize their root architecture by initiating primordia and influencing growth of primary roots or lateral roots (LRs). Root architecture results from the integration of genetic programs governing root growth patterns and environmental factors which affect signaling pathways. We review here recent knowledge acquired mainly in *Arabidopsis thaliana* on primary root and LR development and the impact that different environmental constraints (water, phosphate, nitrate, and sulfate) have on root growth and development. Since Arabidopsis is unable to develop specific organogenesis resulting from symbiotic interactions, we also discuss recent molecular data on the analysis of the nitrogen-fixing symbiotic nodules and their influence on root architecture in legumes. Finally, molecular analysis of the role of noncoding RNAs in environmentally activated signaling pathways will be discussed. These RNAs are emerging as crucial regulators of differentiation and adaptation to environmental conditions.

ABBREVIATIONS

ABA	abscissic acid
advR	adventitious root
BR	brassinosteroids
CC	cortical cell
CK	cytokinin
IC	initial cell
LR	lateral root
miRNA	microRNA
N	nitrogen
nat-siRNA	natural antisense-mediated siRNA
npc RNA	nonprotein coding RNA
P	phosphate
QC	quiescent center
QTL	quantitative trait locus
RAM	root apical meristem
ROS	reactive oxygen species
S	sulfur
siRNA	small interfering RNA
*ta*siRNA	*trans*-acting siRNA

I. INTRODUCTION

Plant development after germination is essentially derived from stem cells localized in two apical regions formed during embryogenesis, the shoot and root apical meristems. This particular characteristic allows plants, which are sessile organisms, to adapt their morphology and consequently organ development to environmental conditions. The root system, which

shows indeterminate growth, plays a crucial role in the survival of land plants under a wide variety of conditions. It assures two main functions: the anchorage to the soil and the exploration thereof for water and mineral nutrients. The root system has therefore a major impact on crop yield and productivity (Lynch, 1995). Moreover, the root is a remarkable example of developmental plasticity: its spatial configuration (number and length of lateral organs), so-called architecture, varies greatly, depending on the plant species, soil composition, and, particularly, on water and mineral nutrients availability. Thus, extensive morphological differences (in size, number, and distribution of lateral root organs) are observed in genetically identical plants cultivated under different nutritional conditions (Lopez-Bucio *et al.*, 2003). An optimal adaptation of root architecture to the soil allows plants to recover efficiently critical resources and increase their ecological fitness when these resources are limited. Understanding the molecular mechanisms governing such developmental plasticity is therefore likely to be crucial for crop improvement in sustainable agriculture.

Root architecture is under the coordinated control of both genetic endogenous programs regulating growth and organogenesis and the action of abiotic and biotic environmental stimuli. The mature root system therefore results from the integration of intrinsic and extrinsic signals (Malamy, 2005). Their interactions however complicate the dissection of specific transduction pathways involved in root growth and development. Such complex traits likely depending on multiple genes may be efficiently analyzed through quantitative genetics. For instance, in the model plant *Arabidopsis thaliana* and in maize, a largely cultivated cereal species, quantitative trait loci (QTL) linked to root architecture have been identified (Mouchel *et al.*, 2004; Tuberosa *et al.*, 2002a,b).

In this chapter, we discuss the influence of the soil environment on root growth and differentiation through its action on existing and *de novo* meristems. First, we will briefly describe the Arabidopsis model root system and its main features: the root apical meristem (RAM) and lateral roots (LRs). In the wild, plant roots are surrounded by microorganisms in the rhizosphere that can modify their architecture. Unfortunately, *A. thaliana* is not able to form symbioses, although root symbiotic associations are essential to more than 80% of higher plants (Hirsch and LaRue, 1997). Hence, a second part of this chapter will be dedicated to the symbiotic associations of legumes with bacteria, collectively called rhizobia. These bacteria modify the root system by inducing the formation of new meristems which form root nodules that are able to fix nitrogen (N). This allows legumes to grow in N-poor soils (Crespi and Galvez, 2000; Stacey *et al.*, 2006). In contrast, mycorrhizal associations between fungi and plant roots allowing the expansion of the

explored soil volume will not be discussed here as they do not imply the formation of new meristems. Recent reviews are available on this subject (Brachmann, 2006; Gianinazzi-Pearson *et al.*, 2007; Graham and Miller, 2005; Harrison, 2005; Wang and Qiu, 2006) as these associations may have been critical for the colonization of the land by plants early in evolution and have a major impact on root metabolism and architecture. Furthermore, we will not describe here the effects of plant growth-promoting rhizobacteria (PGPR) in promoting LR development since an excellent review in this issue is dedicated to this topic (Molina-Farero *et al.*, 2007). In a third part of this chapter, we will emphasize on the impact of certain soil resources and their availability on the modification of the root system growth. Finally, we will focus on the recent work on RNA-mediated posttranscriptional regulation, which may be crucial in root differentiation, auxin signaling as well as biotic and abiotic interactions, to further apprehend the diverse mechanisms involved in the formation of a root system.

II. THE ROOT SYSTEM AND THE MODEL *A. THALIANA*

Arabidopsis displays a typical allorhizic root system: the primary root is derived from the embryonic root and the development of LRs is initiated from a specific set of cells located in the pericycle of the primary root. Adventitious roots (advRs) can also appear, under particular culture conditions, differentiating from pericycle cells at the hypocotyl–root junction (Sorin *et al.*, 2005). Arabidopsis roots, like most monocots and dicots, comprise three zones: (1) the distal root apex, consisting of the root cap that protects the underlying RAM, where cells divide actively; (2) an elongation zone above the RAM, where cells expand mainly in a longitudinal direction; and (3) a differentiation zone.

Roots are composed of concentric cell layers originating from the RAM (Fig. 1). The Arabidopsis epidermal cell layer (the most external) presents a specific pattern of root hair distribution, with a defined alternation of atrichoblast and trichoblast cell files (corresponding to non-hair-forming and hair-forming cells, respectively) (Dolan and Costa, 2001).

The two inner layers, called cortex and endodermis, which envelop the stele, consist each of a single cell file (Benfey and Scheres, 2000). Even though the Arabidopsis root patterning is generally conserved, many variations in root anatomy exist. For example, in legume roots, the epidermal cell files show no specific root hair patterning and the cortex consists of three to five cell layers, usually defined as outer, middle, and inner cortex (Gage, 2004).

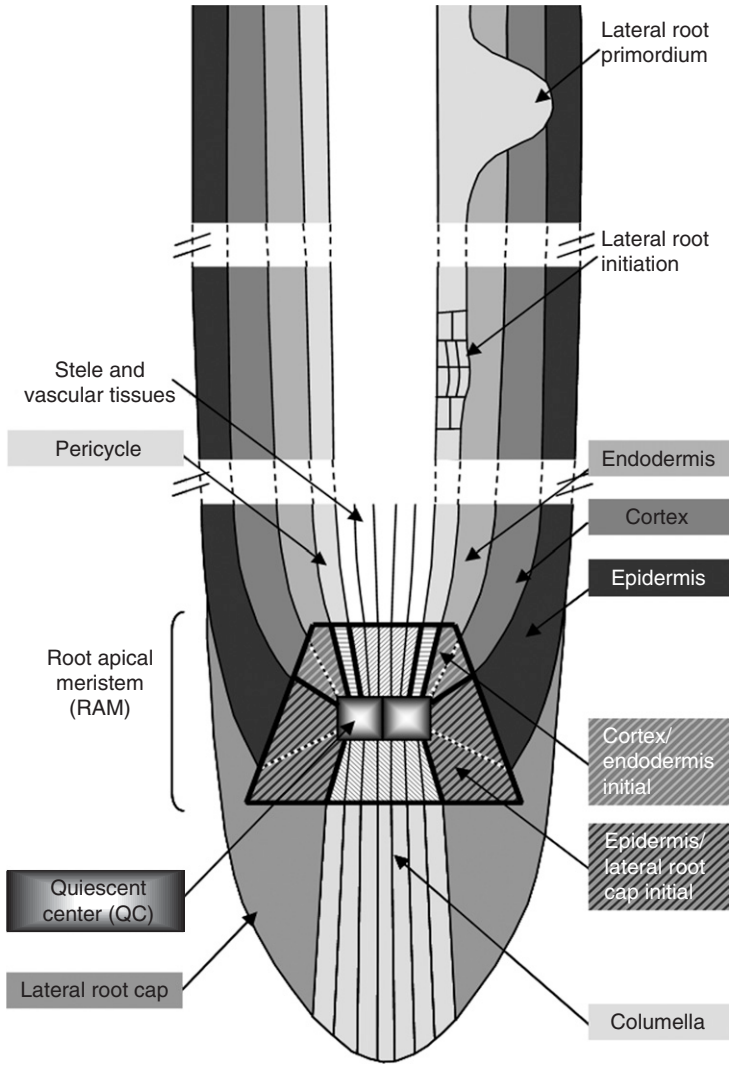

Fig. 1. Schematic representation of primary root cell lineage and LR formation.

A. THE RAM: ESTABLISHMENT AND PATTERNING

The RAM of angiosperms comprises a slowly dividing quiescent center (QC), which is surrounded by mitotically active initial cells (ICs) that give rise to the different cell types constitutive of root tissues and therefore could be considered as stem cells (Fig. 1; Benfey and Scheres, 2000). Plant and animal stem cells develop in a microenvironment, the stem cell niche, where they can be auto-maintained in a nondifferentiated state through the action of diverse

signals (Singh and Bhalla, 2006). In Arabidopsis, the QC consists of 4 cells surrounded by 1 IC layer, whereas in maize, 800–1200 cells may compose the QC, surrounded by several hundred ICs (Feldman, 1994). After each IC division, one daughter cell leaves the RAM, becomes isolated from the stem niche signal(s), and then starts differentiation. To better understand QC function, Nawy *et al.* (2005) used microarrays to determine the expression pattern of its four cells. They first generated a transgenic line expressing a marker under the control of a *cis*-regulatory sequence belonging to the gene encoding the MADS box transcription factor AGL42, expressed in the QC. Using cell-sorting of root protoplasts, cells expressing this construct were used in a transcription profiling experiment that demonstrated the enrichment of 290 genes belonging to 3 major functional categories: (1) hormonal signaling [auxin: 5 genes, gibberellin (GA): 3 genes, and brassinosteroid (BR): 1 gene]; (2) transcription factors (37 genes); and (3) metabolism (63 genes). The absence of phenotypes for mutants affected in 11 of the QC-enriched transcription factors suggests functional redundancies between them, likely to assure root growth and survival.

RAM specification occurs very early in embryo development with differentiation of the hypophysis, the apical cell of the suspensor (Benfey and Scheres, 2000). Auxin appears to be essential for this process as many auxin-related mutants, such as *monopteros* (*mp*), *bodenlos* (*bdl*), and *auxin transport inhibitor resistant 1* (*tir1*) and related *tir1/afb1–3* (*auxin signaling F-box gene 1, 2, and 3*) quadruple mutant, are unable to specify the hypophysis and then to form the embryonic RAM. The auxin flux coming from the apical region of the embryo into the hypophysis leads to TIR1 (and related redundant AFBs) pathway activation and induction of auxin-response genes such as *PIN* genes (coding for auxin efflux carriers), whose products will increase auxin transport and accumulation into the hypophysis to further differentiate this cell (Benkova *et al.*, 2003). After division, the hypophysis generates the QC and part of the root cap. RAM differentiation is under auxin control and involves a complex network of interactions in order to maintain the stem cell niche in the distal part of the root (Aida *et al.*, 2004).

The RAM has two functions: (1) determination of the root patterning, through IC stereotyped divisions, leading to the formation of the different root cell files and (2) auto-maintenance of stem cells to allow later postembryonic root growth. Two GRAS transcription factors, SCARECROW (SCR) and SHORT ROOT (SHR), have been associated with RAM maintenance: indeed, root growth is delayed in *scr* and *shr* mutants due to the lack of one IC formation, leading to the absence of endodermal cell files (Di Laurenzio *et al.*, 1996; Helariutta *et al.*, 2000; Scheres *et al.*, 1995). Although SHR proteins control *SCR* expression, QC function cannot be completely rescued when the SCR protein is overexpressed in an *shr* background. Levesque

et al. (2006) have identified eight potential targets for SHR using microarrays analyses. Thus, SHR not only controls *SCR*, but certainly acts on other genes to regulate IC differentiation.

B. RADIAL ORGANIZATION OF ROOT TISSUES

Arabidopsis roots can be viewed as a set of concentric cylinders. As mentioned earlier, the epidermal cells form trichoblasts and atrichoblasts. With respect to the position of the neighboring cortical cells (CCs), contact of one epidermal cell with only one CC would lead to an atrichoblast fate, while contact with two CCs would lead to a trichoblast fate (Berger *et al.*, 1998). A whole regulatory network of transcription factors and, more recently, chromatin organization (at least at some loci like *GLABRA 2*) have been involved in signaling the positional information defined by CCs (Bernhardt *et al.*, 2003; Costa and Shaw, 2006). The cortical and endodermis cell files originate from the asymmetrical division of a single IC. The SHR and SCR transcription factors are involved in this specification event, and SHR synthesized in the stele may diffuse into the endodermis to regulate *SCR* expression. This movement may be linked to cell specification in the radial axis of the root (Gallagher *et al.*, 2004).

C. LR ORGANOGENESIS

In dicots, the root system is constituted by the primary root and several orders of LRs, which are produced throughout the plant's life. Root system architecture is dependent on the number and size of LRs. LR development (Fig. 1) can be divided in different steps: primordium initiation and development, emergence, and meristem activation. LR initiation is the key element for LR development. It occurs strictly acropetally; for example, a primordium is always initiated in a more distal root portion relatively to already initiated LRs and *de novo* initiation is not possible between two LRs primordia or two mature LRs. Moreover, branching capacity may be accession specific (Dubrovsky *et al.*, 2006).

Pericycle founder cells, from which the LRs originate, are peculiar cells that retain the ability to dedifferentiate and divide—a characteristic of stem cells—even after leaving the RAM (Beeckman *et al.*, 2001; DiDonato *et al.*, 2004; Dubrovsky *et al.*, 2000). This particular cell population accounts mainly for the extensive developmental plasticity of the root and may be responsive to both an endogenous control and environmental cues. How the competence of the founder cells is determined remains still unknown. In Arabidopsis, the root primordium originates from at least three founder

cells (Fig. 1) undergoing first anticlinal divisions in front of protoxylem poles (Malamy and Benfey, 1997a,b). This event is essential to LR initiation: *alf4* mutant (*aberrant lateral root formation 4*), which is blocked in the LR initiation, has lost its capacity to maintain pericycle cells in a mitotically active state (DiDonato *et al.*, 2004); this has been nicely shown using the *CycB1;1* marker gene (only expressed around the G2/M cell cycle transition) (Fukaki *et al.*, 2002). As well, the dominant mutation *slr-1* (*solitary root-1*) affected in IAA14 (a member of the AUX/IAA protein family) cannot develop LRs due to a lack of early cell divisions (Fukaki *et al.*, 2002).

Unlike Arabidopsis, LR primordia of other angiosperms arise from periclinal divisions, and sometimes in front of protophloem pole (Mallory *et al.*, 1970). After the primordium has been formed inside the parental root, cell elongation is responsible for its emergence outward. The LR meristem seems identical to the embryonic RAM. Mutant analyses indeed revealed that abnormalities found in embryonic roots were also found in LR primordia (Helariutta *et al.*, 2000; Wysocka-Diller *et al.*, 2000).

III. ROOT GROWTH IN THE SOIL ENVIRONMENT

Root growth in the soil is regulated by endogenous signals that maintain RAM activity and patterning as well as contribute to the generation of new LRs. Among them, auxin plays a crucial role, although other hormones contribute to the overall root architecture. We will emphasize here on the role of hormone signals in this regulation based on molecular genetic studies mainly in Arabidopsis. However other signals, such as the redox status, may also play significant roles in root growth and development. For example, the RAM is highly sensitive to glutathione levels: in the *root meristemless 1* mutant (*rml1*), which presents a short root phenotype, the mutated protein catalyzes the first step of glutathione biosynthesis, and the root growth defects have been correlated with a very low level of glutathione (Vernoux *et al.*, 2000). Combined analyses of different accessions or mutants affected in root architecture under various environmental conditions allowed to identify several hormone signaling pathways and even QTL that regulate LR size and distribution (De Smet *et al.*, 2006; Fitz Gerald *et al.*, 2006; Loudet *et al.*, 2005).

A. ENDOGENOUS SIGNALS REGULATING ROOT GROWTH

Auxin, the major determinant of root growth, actively participates in embryonic and postembryonic root development as well as gravitropism. It can be synthesized in seedlings either in the aerial parts of the plant or at the tips of

primary roots and LRs (Ljung *et al.*, 2005). The auxin fluxes, which are under a variety of controls involving *PIN* as well as *AUX1* influx carrier genes, converge to the RAM (Friml *et al.*, 2006). In all the species studied so far, inhibition of auxin transport leads rapidly to a decrease in primary root growth (Blilou *et al.*, 2005). In certain Arabidopsis *pin* mutants, auxin distribution is altered and root growth is slightly affected, suggesting functional redundancies between PIN proteins (Friml *et al.*, 2006). To further characterize the role of five of these genes during growth and root patterning, Blilou *et al.* (2005) used various combinations of double, triple, and quadruple *pin* mutants (*pin1* to *pin4* and *pin7*). This elegant work confirmed that PINs collectively control auxin distribution in the root and that the circulating flow of this hormone regulates meristem size. Moreover, this study showed that cell division and elongation are controlled by modulation of auxin distribution. The AUX1 (AUXIN RESISTANT 1) influx carrier is also involved in the regulation of auxin fluxes at the root tip and has been mainly described as critical in the root cap as well as in the epidermis to allow root gravitropic responses (Bennett *et al.*, 1996; De Smet *et al.*, 2007; Sieberer and Leyser, 2006; Swarup *et al.*, 2005). The major role of auxin in LR initiation and development has been known for years; indeed, both an exogenous application or an endogenous overaccumulation of auxin via plant transformation cause an increase in LRs number (Boerjan *et al.*, 1995; Celenza *et al.*, 1995). Furthermore, a disturbed polar auxin transport between the stem and the primary root completely blocks the initiation of LRs (Reed *et al.*, 1998). Several mutants altered in the transport, signaling, or homeostasis of auxin are also affected in LR initiation and emergence (Casimiro *et al.*, 2003; De Smet *et al.*, 2006). For instance, the *aux1* mutant is affected in promotion of LR development and their positioning along the parental root (De Smet *et al.*, 2007; Marchant *et al.*, 2002). AUX1 action in LR cap and/or epidermis induces priming of pericycle cells in the meristem. Moreover, specific PIN members may be linked to LR organogenesis (Benkova *et al.*, 2003). In addition, *alf3* mutants (*aberrant lateral root formation 3*) do not seem able to activate the growth of the LR meristem. Although the function of *ALF3* is not known, the wild-type phenotype can be restored by an exogenous supply of auxin, suggesting a role for this gene in hormone production or accumulation (Celenza *et al.*, 1995). Finally, AUX/IAAs (a 29 members' multigene family) and ARFs (23 members) show a large diversity of expression patterns in different root domains and root cell types, likely determining the global action of auxin on root development (Remington *et al.*, 2004). *SOLITARY-ROOT/IAA14* as well as *NPH4/ARF7* and *ARF19* and their recently identified direct regulatory targets LBD16/ASL18 and LBD29/ASL16 (LATERAL ORGAN BOUNDARIES-DOMAIN/ASYMMETRIC LEAVES2-LIKE) have been involved in the

control of LR initiation, and transcriptome analyses revealed that several *ARF*s and *AUX/IAA*s are among the earliest activated genes during LR initiation (Fukaki *et al.*, 2005; Okushima *et al.*, 2007; Vanneste *et al.*, 2005; Wilmoth *et al.*, 2005).

AUX/IAA genes show a very early response to auxin and encode proteins present at low concentrations, with a short half-life, generally localized in the nucleus where they act as negative regulators of auxin-response genes (Abel *et al.*, 1994). Notably, AUX/IAA can form heterodimers with ARFs, transcription factors that recognize, in a hormone-independent manner, the auxin-response elements (AREs) present in auxin-inducible genes (Ulmasov *et al.*, 1999). Indeed, BDL/IAA12 and MP/ARF5 antagonistic proteins have been shown to interact *in vivo* in the embryo (Berleth and Jürgens, 1993; Hamman *et al.*, 2002). AUX/IAA-ARF dimers subsequently repress transcription of these genes. Fixation of auxin on the F-box protein then stimulates interaction between SCF^{TIR} E3 ubiquitin ligase complex and the AUX/IAA, via the recognition of the "degron" motif. The ubiquitinated AUX/IAA proteins are finally degraded by the 26S proteasome and the promoter-associated ARFs, thus relieved from inhibition, promote transcription of the downstream genes. TIR1, inside the SCF^{TIR} complex, corresponds to one of the long-awaited auxin receptors (Dharmasiri *et al.*, 2005; Kepinski and Leyser, 2005). Recently, some ARFs and certain auxin-related F-box have been shown to be regulated by microRNAs (miRNAs) or *ta*siRNAs (*trans*-acting siRNAs) posttranscriptional mechanisms, and this regulation is crucial for postembryonic root development (see Section V).

BRs play multiple roles in cell elongation, senescence, photomorphogenesis, and stress responses in plants (Nemhauser and Chory, 2004). A link between auxin and BR signaling pathways has been described, and microarray data analysis also strongly suggests that both pathways converge to regulate the expression of similar target genes (Goda *et al.*, 2004; Nemhauser *et al.*, 2004). The nuclear protein BRX (BREVIS RADIX), which is involved in the regulation of transcription, seems to be one of the cross talk elements between these two hormonal pathways (Mouchel *et al.*, 2004, 2006). The *brx* mutant is strongly affected in its root growth, with few and small root cells as well as a smaller RAM than the wild type (Mouchel *et al.*, 2004). As already mentioned, a reduced meristem size could be a consequence of an altered auxin transport (Blilou *et al.*, 2005). Transcriptome analysis showed that up to 15% of the transcriptome is affected in *brx* roots. Notably, the expression of three genes [*PIN3*, *PIN4*, and *PGP4* (*ATP-BINDING CASSETTE P GLYCOPROTEIN*)] involved in auxin flow at the root tip is reduced. Moreover, the transcripts corresponding to *CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARF* (*CPD*) are barely detectable

in the *brx* mutant roots. The CPD enzyme catalyzes a limiting step in the biosynthesis of brassinolide, the predominant BR in Arabidopsis. This study shows that during root growth, BRX is responsible for both BR level regulation and auxin signaling (Mouchel *et al.*, 2006).

Cytokinins (CKs) are involved in many developmental processes and in cell division control. CK synthesis occurs mainly in root tips, even though the expression of isopentenyltransferases (IPTs), a key biosynthesis enzyme, has been detected in other plant organs (Miyawaki *et al.*, 2004). Overexpression of IPTs or CKX (CYTOKININ OXIDASE) involved in CK degradation leads to modifications in the CK pool, correlated with root developmental defects. CKX-overexpressing plants have indeed an increased root length and more LRs (Werner *et al.*, 2001, 2003). Recently, it has been observed that the CK receptor CRE1/AHK4 (CYTOKININ RESPONSE 1/ HISTIDINE KINASE 4) and many response regulator (RR) genes are mainly expressed in roots (Higuchi *et al.*, 2004; Mason *et al.*, 2004). A particular mutant allele affecting the *CRE1/HK4* gene, *wooden leg* (*wol*), showed a drastic short root phenotype associated with specific defects in phloem differentiation (Scheres *et al.*, 1995). Triple mutants of the *ahk2/ahk3/ahk4* CK receptors show a similar phenotype, whereas an *ahk2/ahk3* mutant has increased root length and LR number (Higuchi *et al.*, 2004; Nishimura *et al.*, 2004; Riefler *et al.*, 2006). These results suggest that apart from CRE1, other CK receptors may play overlapping functions in root growth. Different combinations of mutants affecting other CK signaling elements (AHP, for histidine phosphotransfer proteins and RRs) also confirmed the crucial role of CK in root architecture (both on primary root growth and LR formation), even though the precise developmental stage where they are involved remains to be determined (Ferreira and Kieber, 2005; Mason *et al.*, 2005; Rashotte *et al.*, 2006; To *et al.*, 2004). CK effects on meristematic activity and in vascular bundles differentiation may be responsible for the described defects in root architecture.

Ethylene also plays a major role in root growth eventually through its interactions with auxin signaling (Souter *et al.*, 2004; Stepanova *et al.*, 2005). The *ethylene overexpression 1* (*eto1*) mutant plants overaccumulate ethylene, have an increased sensitivity to ethylene, and display a shorter primary root than wild-type plants. Analysis of *polaris* (*pls*) mutants, which also display a short root phenotype, has underlined a possible interplay between auxin and ethylene signaling pathways. The auxin-regulated *PLS* gene encodes a 36-amino acids-long peptide, essential for proper auxin transport and thereby root growth. This peptide inhibits ethylene signaling, leading to an arrest of the cytoskeletal dynamics required for root growth (Chilley *et al.*, 2006).

The role of other hormones such as abscisic acid (ABA) and GAs in root development will be described in relation to environmental stresses (see Section IV).

B. THE PECULIAR LEGUME ROOT SYSTEM AND ITS SYMBIOTIC INTERACTIONS

In legumes, the soil environmental conditions together with the symbiotic interactions are the major determinants of root architecture. Legume roots can develop two types of secondary root organs: LRs and N-fixing nodules. The latter organs result from the symbiotic interaction with soil bacteria collectively known as rhizobia. These bacteria colonize the root surface, attach to root hairs, and induce their deformation and curling as well as a series of rapid changes in root hair cells, such as calcium spiking, depolarization of the plasma membrane, and gene expression (Oldroyd and Downie, 2004). Concomitantly to rhizobial infection, pericycle cells are transiently stimulated for division. Then, cortex cells divide, usually in front of a protoxylem pole close to the infection point (Timmers *et al.*, 1999). These actively dividing CCs form most of the nodule primordium, wherein large amounts of amyloplasts accumulate. At the root surface, rhizobia penetrate into root hairs through plant-derived infection threads. Infection threads progress intracellularly through the outer cortex, ramify, and finally penetrate the nodule primordium cells. A differentiation process is then initiated heralded by cell enlargement in both partners. Bacteria differentiate into specific N-fixing forms called bacteroids, surrounded by a peribacteroid membrane, which are released from infection threads into the cytoplasm of the enlarged plant cells forming symbiosomes. In parallel to bacteroid differentiation, the nodule primordium, comprising a persistent or transient meristem (according to the plant species), develops into a mature nodule (Brewin, 1991).

The organogenesis of legume nodules requires a precise spatiotemporal expression of specific genes during the different stages of the symbiotic interaction. Analyses of plant signaling pathways involved in the early stages of this developmental process have been carried out, mainly based on genetic approaches and high-throughput gene expression studies (Stacey *et al.*, 2006). A model for the early stages of the symbiotic interaction leading to nodule organogenesis has been proposed (Geurts *et al.*, 2005). Nodules and LRs share several aspects of their development, even though they have divergent developmental origins (Hirsch and LaRue, 1997; Mathesius *et al.*, 2000). LRs and nodule primordia are formed primarily from different tissues, pericycle and cortex, respectively (Brewin, 1991; Hirsch, 1992). Thus, even though the same root tissue layers are involved, they have different relative contributions

to the respective primordia. Patterns of IC divisions are divergent between both lateral organs, and furthermore, legume nodules lack a root cap and have a peripheral vasculature.

Several legume mutants affected in genes with a dual function in nodule formation and root development were recently identified, such as *latd/nip* (*lateral root organ-defective*) and several hypernodulating mutants (*har1*, *hypernodulation and aberrant root formation*; *sunn*, *supernodule number*; *nts382*, *nitrate sensitive 382*; *skl*, *sickle*), suggesting the existence of common regulatory pathways between these two root-derived organogeneses (Bright *et al.*, 2005; Day *et al.*, 1986; Penmetsa and Cook, 1997; Penmetsa *et al.*, 2003; Veereshlingam *et al.*, 2004; Wopereis *et al.*, 2000). Other mutants such as *crinkle* and *astray* are additionally affected in other plant organs (Nishimura *et al.*, 2002; Tansengco *et al.*, 2003). The *Medicago truncatula latd* main root grows normally few days after germination, later it stops and a strong inhibition of LR formation is observed (Bright *et al.*, 2005). The disorganized *latd* LRs lack a visible root cap and nodule primordia remain small, white, and undifferentiated. The *LATD* gene seems therefore required for the function of three root-derived meristems (e.g., primary root, LR, and symbiotic nodule). Hypernodulating or supernodulating mutants are affected in autoregulation, a systemic feedback mechanism negatively controlling the final number of nodules formed in legume root systems (Caetano-Anollés and Gresshoff, 1991). These negative autoregulatory mechanisms may also affect the regulation of other root meristems (primary roots and LRs) since LR density and certain hormonal responses related to LR formation are perturbed in at least some of these mutants such as *har1* (Krusell *et al.*, 2002; Wopereis *et al.*, 2000). Consequently, the whole architecture of legume roots in symbiotic or nonsymbiotic growing conditions may be at least partially controlled by the same genes.

IV. CHANGING ROOT ARCHITECTURE: ADAPTIVE RESPONSES TO THE SOIL ENVIRONMENT

Under natural culture conditions, modifications of soil composition occur generally in a slow and progressive manner, thus allowing plants to set up an adaptation strategy. Generally, after perception of abiotic stresses such as mineral deficiencies or water stress, both local and systemic signals maybe integrated in these adaptive responses. In contrast, the widespread experimental laboratory conditions usually rapidly impose a strong stress to the plant which produces major changes on gene expression. From these results, extrapolations to real field conditions need to be prudently analyzed.

The main abiotic stresses affecting root architecture are water stress—water deficit or high water retention in the soil—and deficiencies in essential mineral nutrients such as phosphorus, nitrogen, and sulfur. To cope with these deprivations, plants increase their uptake ability, as they modify the nutrients solubilization in the soil by excreting organic compounds or enzymes, and also adapt their root architecture. New LR formation and/or LR growth as well as the differentiation/elongation of root hairs lead to a considerable increase of the overall absorption surface. In Arabidopsis, identification of mutants affected either in the biosynthesis, perception, or signal transduction of hormones on one hand, and transcriptome studies on the other hand have shed light on hormone-regulated target genes and developmental processes involved in root growth and development. Nevertheless, much less is known on the cross talk or overlap between these different signaling pathways during adaptive developmental responses to the environment. Several excellent reviews, dealing with the modifications of Arabidopsis root architecture in response to environmental conditions, have been recently published (Lopez-Bucio *et al.*, 2003; Malamy, 2005). We will thus further discuss only recent relevant results in this research field.

Between the application of a given stress and the following root morphological adaptations, early events such as modifications of gene expression can be monitored. For example, a deficiency in essential inorganic nutrients (phosphate, nitrate, and sulfate) induces genes encoding the corresponding high-affinity transporters (Lopez-Bucio *et al.*, 2003). In addition, reactive oxygen species (ROS) are produced; it is known that ROS act as signal molecules in all types of stresses. In fact, ROS fluctuations in time and space can be interpreted as signals to regulate growth, development, cell death, and stress responses (Foreman *et al.*, 2003; Gechev *et al.*, 2006). *In fine*, the particularity of the biological response (e.g., a modification in root architecture) to a given constraint appears to be dependent on numerous factors: the production site, nature and intensity of signals in response to stress (such as ROS), the developmental and nutritional state of the plant, and also the modifications undergone by the plant before the stress occurred [e.g., stress acclimation (Malamy, 2005; Mittler, 2006; Shin *et al.*, 2005)].

A. WATER AVAILABILITY AND THE OSMOTIC POTENTIAL OF THE MEDIUM

Acidity and concentration of inorganic nutrients in the soil or sucrose concentration *in vitro* not only determine the osmotic potential of the substrate but also influence plant nutrition. All these parameters are rarely taken into account when plants are cultivated *in vitro* (e.g., in the presence of various concentrations of inorganic nutrients on a medium supplemented

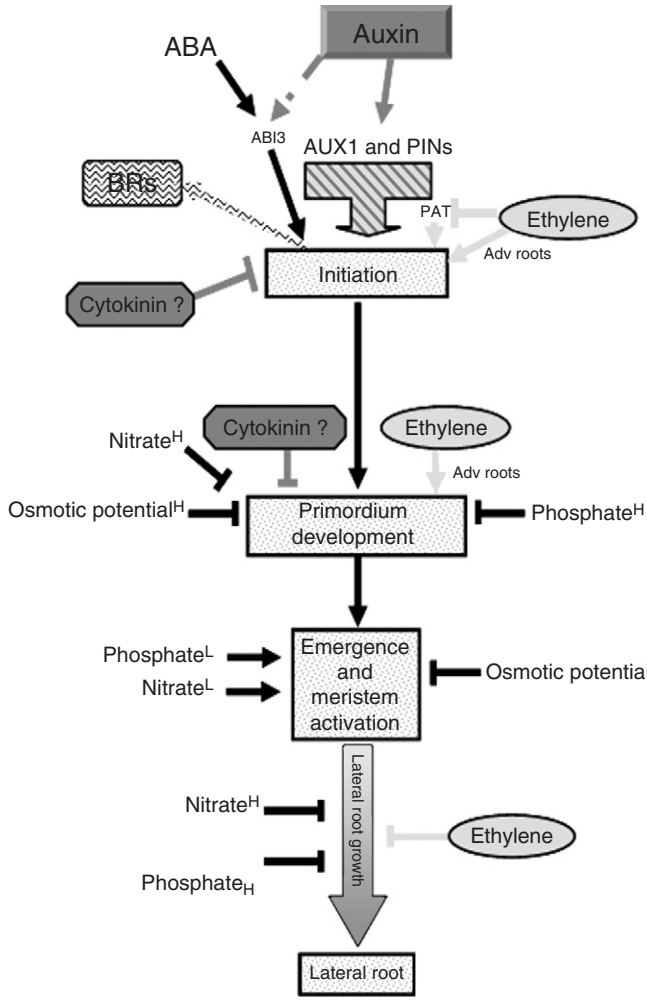

Fig. 2. Environmental and endogenous factors affecting LR development. ^{H}, high concentration; ^{L}, low concentration; BR, brassinolide; PAT, polar auxin transport (shoot root).

with 1–4.5% sucrose). *In vitro*, Arabidopsis roots are very sensitive to the osmotic potential of the medium; under certain conditions, the undergone osmotic stress resembles the one provoked by a water deficit (Deak and Malamy, 2005). LR formation is repressed by an osmotic stress, and a reverse correlation exists between the strength of the osmotic potential and LR growth. Osmotic potential is thought to affect the number of fully developed LRs by acting on primordia development, emergence, and meristem activation rather than the initiation step (Fig. 2; Deak and Malamy, 2005).

However, van der Weele *et al.* (2000) reported a decrease in LR number correlated with a disturbed step of LR initiation during an osmotic stress caused by PEG. During a progressive drought stress, newly formed LRs exhibit a particular phenotype: roots are short, tuberized, do not form hairs, and accumulate starch (Vartanian, 1981).

ABA plays a critical role in the plant response to water stress. In ABA-deficient mutants (*aba*), the root system is largely developed. In wild-type plants, exogenous ABA treatments lead to a dormancy of the newly LRs formed, a phenomenon also noticed during water stress (Deak and Malamy, 2005; De Smet *et al.*, 2003). This particular LR dormancy could have an essential adaptive role: to allow a rapid recovery of root growth and absorption functions once the environmental conditions are favorable again. The relationship between LR dormancy and tolerance has just been demonstrated using a genetic approach: the *dig3* mutant (*drought inhibition of lateral root growth 3*), in which LR growth is not inhibited by ABA, is in fact much more sensitive to stress than the wild type (Xiong *et al.*, 2006). Still, this mutant displays a classical response to osmotic stress, as marker genes (generally under the control of ABA) are correctly expressed. The *DIG3* locus does not bear any known stress-related gene, suggesting that DIG3 could be a component of a yet unknown regulation pathway. The same type of interrelation—growth inhibition by ABA and stress tolerance—has been observed in plants overexpressing the RGS1 protein (REGULATOR OF G-PROTEIN SIGNALING) that intervenes in the G-protein–mediated signal transduction pathway (Chen *et al.*, 2006). Vartanian *et al.* described that *aba1* and *abi1* mutants display a decreased number of "short roots" compared to wild type in response to progressive drought stress. This indicates that ABA plays a promoting role in drought stress-induced rhizogenesis, in other words blocks the expansion of the root system. However, no changes were found in *abi2* and *abi3* mutants (Vartanian *et al.*, 1994).

The involvement of ABA, the stress-related hormone, in modifications of the root system further underlines its relationship with auxin signaling. The overlap between both signaling pathways had already been noticed while studying the *abscisic acid insensitive 3* (*abi3*) mutant, which has a subtle LR phenotype and is less responsive to auxin treatments (Brady *et al.*, 2003). This interdependence may be linked to the ability of the transcription factor ABI3, at least in common bean, to bind as efficiently to promoter sequences of both ABA- and auxin-inducible genes (Nag *et al.*, 2005). As well, the analysis of several ethylene mutants, in particular *etr1* (*ethylene response 1*) and *ein2* (*ethylene insensitive 2*), has shown that a functional ethylene signaling pathway is required for normal root growth in response to ABA (Beaudoin *et al.*, 2000; Ghassemian *et al.*, 2000).

GAs may also be involved in osmotic stress responses. These hormones are known to stimulate plant growth via the degradation of DELLA proteins through the ubiquitination pathway (Fu and Harberd, 2003). These nuclear proteins are also involved in the attenuation of both shoot and root development in response to environmental stress. When four out of five *DELLA* genes (*GAI*, *RGA*, *RGL1*, and *RGL2*) are mutated in Arabidopsis, root elongation is almost no longer affected by salt stress, demonstrating that GAs play a role in root growth under environmental constraints (Achard *et al.*, 2006).

B. WATER EXCESS AND ADVENTITIOUS ROOTING

Like LRs, advRs develop on the hypocotyl from pericycle cells generally contiguous to xylem poles. The appearance of advRs is controlled by environmental conditions such as levels of water retention in the soil, light, and, for a few legume plants, phosphate (P) deficiency (King and Stimart, 1998; Miller *et al.*, 2003). Auxin plays, as well, a preponderant role in the formation of this particular root type since the *superroot 1 and 2* (*sur*) mutants, which spontaneously produce advR, overaccumulate auxin (Boerjan *et al.*, 1995). However, in certain cases, a role of ethylene in this phenomenon cannot be excluded. Indeed, in water-imbibed soils, this gas diffuses less efficiently and is more accumulated in immersed roots. This overaccumulation may block the auxin flow in specific cells and thus leads to advR formation (Aloni *et al.*, 2006). The scaffold protein RACK1A (RECEPTOR FOR ACTIVATED C KINASE 1A) could also be a part of this signaling pathway as the corresponding mutant is highly impaired in adventitious and LR formation (Chen *et al.*, 2006). The lack of RACK1A function may affect many hormone signaling pathways in Arabidopsis, notably auxin sensitivity. Sorin *et al.* (2005) have correlated the inability of allelic series of *ago1* mutants to form advRs with an accumulation of the auxin-responsive factor *ARF17*. This gene is posttranscriptionally controlled by *MIR160*, a regulation that is perturbed in these *ago1* mutants (Mallory *et al.*, 2005). However, *ago1* null mutants display strong pleiotropic phenotypes as AGO1 is a major player of the posttranscriptional regulation mediated by miRNAs in all tissues (see Section V).

A multigenic control of adventitious rooting has been revealed by characterizing QTLs linked to this trait in Arabidopsis and several tree species (Han *et al.*, 1994; King and Stimart, 1998; Marques *et al.*, 1999).

C. NUTRIENT AVAILABILITY

Plants have set different strategies to cope with inorganic nutrient deficiencies. P deficiency induces P remobilization from macromolecules and/or modifications of root architecture in order to increase the plant's uptake capacity (Raghothama, 1999). On a P-deprived medium, Arabidopsis plants adapt their root architecture as the primary root growth stops and numerous new LRs emerge. In addition, numerous root hairs appear, their length being inversely correlated with the P concentration in the medium (Lopez-Bucio *et al.*, 2003). The high root hair number is linked to an increase in differentiation of epidermal cells into trichoblasts (Ma *et al.*, 2001). Comparative analysis of biomasses after cultivating wild-type and *rhd2* (*root hair deficient 2*) mutant plants, unable to form root hairs, on a P-deficient medium, has demonstrated a key role for root hairs in P uptake (Bates and Lynch, 2000). On a P-rich medium, the primary root growth is maintained, whereas LR development is inhibited at the stage of primordium development (Fig. 1). A particular category of phospholipase D (PLD), called PLDξ, is a component of this differential regulation between primary roots and LRs. The PLDξ are indeed involved in the elongation of the primary root, the inhibition of LR elongation, and root hair initiation (Li *et al.*, 2006; Ohashi *et al.*, 2003).

The main hormone influencing these morphological changes in response to P limitation is auxin as changes in its quantitative levels and distribution and/or cell sensitivity to this hormone have been observed (Nacry *et al.*, 2005). Ethylene and CKs could also play a significant role in signaling during P-starvation responses at the whole plant level (Lopez-Bucio *et al.*, 2002; Martin *et al.*, 2000). Indeed, some genes induced by a P deprivation are repressed by exogenous CK treatments (Martin *et al.*, 2000). Moreover, several mutants insensitive to P deficiency and unable to regulate the P-starvation responsive gene *At4* are affected either in AHK4/CRE1 or AHK3 CK receptors (Franco-Zorrilla *et al.*, 2002, 2005; Martin *et al.*, 2000). A particular transcription factor called PHOSPHATE STARVATION RESPONSE1 (PHR1), regulated by sumoylation, is a key component of the P signaling pathway (Miura *et al.*, 2005; Rubio *et al.*, 2001). PHR1 regulates the expression of many genes specifically expressed under P deficiency, such as those involved in lipid or nucleic acids remobilization as well as *MIR399* (see Section V; Bari *et al.*, 2006).

When N distribution in the soil is spatially unequal, plants set a differential root growth. In nitrate-rich soils, LRs are initiated but blocked just before activation of the meristem, whereas in regions deprived of N source, LR growth is increased (Fig. 2; Linkohr *et al.*, 2002). The LR growth arrest is

much less drastic in ABA-insensitive mutants, suggesting that nitrate-induced meristem quiescence of LRs is mediated by ABA (Signora *et al.*, 2001).

Signaling pathways involved in nitrate-regulated responses are being deciphered. Nitrate itself, and not one of its metabolites, is able to stimulate LR initiation. The nitrate transporter NRT2.1 could be either the sensor or a key component of the transduction pathway (Little *et al.*, 2005; Malamy and Ryan, 2001). Recent analyses of *atnrt2.1–1* mutant lines revealed that the amount of nitrate absorbed, and not its external concentration, governs the modifications of root architecture (Remans *et al.*, 2006). The nitrate-inducible transcription factor ANR1, by feedback mechanisms, could be a regulator which determines the intensity of the LR response (Gan *et al.*, 2005; Zhang and Forde, 1998). Finally, as for P deficiency, a transcription factor from the PHR family overexpressed under N deficiency plays presumably a key role in the whole plant response (Todd *et al.*, 2004). Impact of N deficiency on root morphology is strongly modulated by the overall N status of the plant, implicating long-range signaling in modifications of root architecture (Zhang *et al.*, 1999). This could be a consequence of the interaction between nitrate and auxin biosynthesis or transport, as *axr4* mutant is insensitive to the effect of N on LR growth. Nitrate also induces CK accumulation in roots, which could account for part of the nitrate-induced root growth inhibition (Horgan and Wareing, 1980). Furthermore, glutamate, a metabolite involved in N metabolism, is also able to modify the root architecture of Arabidopsis. Among several N metabolites, only L-glutamate can inhibit the primary root growth and affect LR development *in vitro* (Walch-Liu *et al.*, 2006). Hence, N deficiency modulates root architecture through a complex cross talk between hormone signals, N metabolites, and specific N-regulated signaling pathways.

Sulfur (S) uptake is essential for the biosynthesis of sulfured amino acids, cell metabolism, and stress responses (Kopriva and Rennenberg, 2004). In S-deprived conditions, two types of LR modifications have been observed: either an increase in the number of LRs formed locally close to the root tip or a reduction in the overall number of LRs and primordia that emerge from the primary root (Dan *et al.*, 2007; Kutz *et al.*, 2002; Lopez-Bucio *et al.*, 2003; Nikiforova *et al.*, 2003). A decrease in S uptake can always be linked to many metabolic modifications that strongly change the growth of the plant. An S-responsive element (SURE) has been recently identified upstream of several genes encoding S transporters or involved in S uptake (Maruyama-Nakashita *et al.*, 2005). However, no transcription factor able to bind to these sequences has been identified so far.

Genes involved in S uptake such as the one encoding the high-affinity transporter, *SULTR1;2* (*SULPHATE TRANSPORTER 1;2*), are strongly

regulated during an S deprivation (Maruyama-Nakashita *et al.*, 2004). CKs repress *SULTR1;2* expression and alter the expression of S-metabolism genes (Dan *et al.*, 2007). Transcriptome analyses during S deprivation revealed changes in expression of several genes involved in auxin signal transduction (*IAA9*, *IAA17*, *IAA18*, and *IAA28*) or biosynthesis (*NIT3*) (Nikiforova *et al.*, 2003). However, Kutz *et al.* (2002) did not detect any statistically significant modulation of auxin concentration between S-deprived or control whole seedlings. On the contrary, a downregulation of *DR5::GUS* fusions has been observed, suggesting a decrease in auxin level or sensitivity, which is in agreement with the described decrease in LR number (Dan *et al.*, 2007; Nikiforova *et al.*, 2005). Finally, transcripts corresponding to jasmonic acid biosynthesis genes are accumulated during an S deprivation. Interestingly, jasmonic acid controls several key enzymes of S metabolism (Jost *et al.*, 2005).

The different interactions between hormones and abiotic stresses or nutrient deficiencies are schematized in Fig. 2.

D. EFFECTS OF ABIOTIC STRESSES ON LEGUME ROOTS

Several environmental factors such as nitrate or P availability or growth under abiotic stress conditions influence the development of root-derived organs in legumes. The ability of legume roots to interact with symbiotic microorganisms constitutes an adaptation to specific nutrient starvation conditions (e.g., combined N for the N-fixing symbiosis). Nitrate is particularly relevant for legume root architecture as its availability exerts complex effects on root growth, LR formation, and symbiotic interactions (Dazzo and Brill, 1978; Gresshoff, 1993). Indeed, nitrate deprivation represents the major environmental factor that regulates nodulation and most hypernodulating mutants such as *har1* in *Lotus japonicus*, or several *nts* (*nitrate tolerant symbiosis*) mutants in *G. max* are also affected in their nitrate regulation, suggesting that these two pathways are tightly interconnected (Carroll *et al.*, 1985; Wopereis *et al.*, 2000). As well, P availability affects root development and nodulation (Pereira and Bliss, 1989).

Among abiotic stresses, studies involving physiological, molecular, and functional data in legumes have been carried out mainly on salt stress. Increasing salt concentrations in soils leads to marked changes in the root growth pattern of legumes, and also affects the symbiotic N fixation process. Legumes are very sensitive to salt levels in soils, whereas rhizobia are generally much more tolerant (up to 700-mM NaCl) than their respective hosts (Arrese-Igor *et al.*, 1999; del Papa *et al.*, 1999; Lanter *et al.*, 1981; Singleton and Bohlool, 1984). Different steps of the symbiotic interaction and nodule development are affected by salt stress, leading to a reduction in nodule

number and subsequent limited N fixation (Singleton and Bohlool, 1984). Reduced colonization and early rhizobial infection events (such as root hair curling, infection thread formation, and nodule initiation) are particularly sensitive to salt stress (Duzan *et al.*, 2004).

Genes encoding potential regulators of adaptive responses to osmotic and salt stresses, and more particularly putative transcription factors, have been identified (Hasegawa, 2000). In alfalfa, *MsALFIN1* is a salt-inducible transcript that encodes a zinc-finger protein predominantly expressed in roots (Winicov, 1993). Overexpression of this putative transcription factor enhances root growth under control and saline conditions (Winicov, 2000). Another C_2H_2 zinc-finger transcription factor (ZPT2–1) has been involved in the regulation of bacteroid differentiation in *M. truncatula* (Frugier *et al.*, 1998, 2000). Its expression is induced by salt stress, and antisense transgenic lines are impaired in their ability to recover from a salt stress, suggesting that this transcription factor may be involved in nodule and root osmotolerance responses (Merchan *et al.*, 2003).

In several legume species, unlike Arabidopsis, ABA increases LR development (Liang *et al.*, 2007). Several studies have also shown that exogenous ABA application inhibits nodule formation in various legumes (Suzuki *et al.*, 2004). Observation of root hair infection events in *Trifolium repens* revealed that ABA blocks early infection events such as root hair deformation. Moreover, decreasing ABA levels by using specific inhibitors led to an increase in nodule number (Asami *et al.*, 2003). Thus, ABA, similarly to abiotic stresses, could exert a negative control on nodule number and a positive one in LR formation in legumes. Indeed, *latd* mutants are defective in ABA responses and ABA controls root meristem function (Bright *et al.*, 2005; Liang *et al.*, 2007).

V. ROOT GROWTH AND DIFFERENTIATION IN RESPONSE TO ENVIRONMENTAL CONDITIONS: SMALL NONCODING RNAs AS NEW POSTTRANSCRIPTIONAL REGULATORS

Regulatory pathways involved in growth and differentiation have been recently shown to be dependent on a myriad of small noncoding RNAs. miRNAs are noncoding 20- to 24-nt-long RNAs, initially discovered in *Caenorhabditis elegans* as temporal regulators of larvae differentiation, and more recently in mammals and plants (Lagos-Quintana *et al.*, 2001; Lee *et al.*, 1993; Pasquinelli *et al.*, 2000; Reinhart *et al.*, 2000, 2002; Wightman *et al.*, 1993). miRNAs are encoded by particular genes generally present, in

plants, in intergenic regions of the genome. Maturation of the primary transcript, generated by RNA polymerase II, requires the intervention of a particular type III RNase named DICER-LIKE 1 (DCL1) which cuts twice on a hairpin-structured double-stranded RNA (Kurihara and Watanabe, 2004). The mature miRNA is then incorporated in a protein complex, the so-called RISC (RNA-induced silencing complex) that can recognize mRNAs partially complementary to the miRNA nucleotide sequence. This recognition event mediated by the RISC-loaded miRNA leads to the cleavage (as it is generally the case in plants) or the translational inhibition of the target mRNA. Up to now, 43 miRNA families in 71 different plant species have been defined using homology criteria (Zhang *et al.*, 2006). Sequences of certain *MIR* families as well as their targets are highly conserved, suggesting that those *MIRs* may play the same function in different species. Nevertheless, many other *MIRs* are specific to only one or few phylogenetically related species, indicating their rapid evolution. In plants, *MIRs* have been shown to play significant roles notably in the regulation of differentiation and in response to environmental conditions (Mallory and Vaucheret, 2006).

Another class of small RNAs is the siRNAs (small interfering RNAs), initially identified in plants (Hamilton and Baulcombe, 1999). They intervene mainly in two processes: changes in chromatin conformation (e.g., through methylation) and destruction of foreign RNAs such as viral RNAs or aberrant transgene mRNAs (Voinnet, 2005). Plant siRNAs are 21- to 24-nt RNAs generated from long perfectly matched double-stranded RNAs by the action of DCL2 and DCL3 enzymes (Bouche *et al.*, 2006). These siRNAs lead to the extinction, either posttranscriptionally (PTGS for posttranscriptional gene silencing) or transcriptionally (TGS for transcriptional gene silencing), of the gene from which the dsRNA originates. In plants, two other endogenous pathways leading to gene extinction have been described, one mediated by the *ta*siRNAs and one mediated by nat-siRNA (natural antisense-mediated siRNA) (Borsani *et al.*, 2005; Mallory and Vaucheret, 2006). The 21-nt-long *ta*siRNAs are different from the siRNAs due to their action in *trans* on a gene different from the one encoding them (Peragine *et al.*, 2004; Vazquez *et al.*, 2004b). A long nonprotein-coding RNA (npcRNA) is generated from a *TAS* locus, which is cleaved by the action of an miRNA on one or two sites (Axtell *et al.*, 2006). The npcRNA cleavage products are recognized by an RNA-dependent RNA polymerase (RDR6) and matured into a dsRNA, which becomes a substrate of DCL4 producing the 21-nt *ta*siRNAs. The nat-siRNA (only one has been described up to now) is generated in response to a salt stress in Arabidopsis and will be described later (see below) (Borsani *et al.*, 2005).

Mutants affected in miRNA metabolism (biosynthesis, action, and transport as *dcl1*, *ago1*, *hen1*, *hyl1*, *hst1*, *se*) show pleiotropic phenotypes confirming the role of miRNAs in diverse developmental processes (Bollman *et al.*, 2003; Chen *et al.*, 2002; Jacobsen *et al.*, 1999; Kidner and Martienssen, 2005; Vazquez *et al.*, 2004a; Yang *et al.*, 2006). miRNAs action is exerted directly on transcripts coding for genes involved in development (e.g., transcription factors), notably auxin signaling genes such as the ARF transcription factors (Teale *et al.*, 2006). In Arabidopsis, *MIR160* targets *ARF10, ARF16*, and *ARF17* transcripts, whereas transcripts encoding ARF3 and ARF4 proteins are recognized by *ta*siRNAs derived from the *TAS3* loci (see also Section IV; Fahlgren *et al.*, 2006; Mallory *et al.*, 2005; Rhoades *et al.*, 2002; Wang *et al.*, 2005; Williams *et al.*, 2005a). Using experimental approaches that modify the miRNA pairing site in the target transcript without affecting the encoded protein (known as miR-resistant transcripts) and by overexpressing miRNAs (thus reducing target transcript levels), Sorin *et al.* (2005) and Mallory *et al.* (2005) demonstrated the involvement of *MIR160* in the regulation of *ARF17* transcripts during root development and branching. Furthermore, *MIR160*, through its action on *ARF10* and *ARF16* mRNAs, plays a primordial role in root cap differentiation (Wang *et al.*, 2005). Indeed, constitutive expression of *MIR160* inhibits the root cap cell differentiation and results in agravitropic roots. Additionally, mRNAs encoding the NAC1 transcription factor involved in late steps of auxin signal transduction pathway and LR formation are regulated by *MIR164* (Xie *et al.*, 2000). Overexpressing *MIR164* (using an inducible promoter) or an *MIR164*-resistant *NAC1* mRNA leads to a significant decrease in LR number (Guo *et al.*, 2005). Noteworthy, these experiments have been done using very high sucrose concentration which affects root architecture (see Section IV) and a strong promoter mixing both the effects of miRNA cleavage and the misregulation of *NAC1* transcripts. These experiments suggest that the *MIR164*-mediated regulation of NAC1 is involved in LR formation in Arabidopsis.

Bioinformatic predictions on miRNA-target interactions in plants suggest that miRNA-mediated regulation may contribute to plant stress responses (Jones-Rhoades and Bartel, 2004). The first observation that environmental conditions could affect miRNA expression was done on Arabidopsis plants grown on a sulfate-deprived medium. These plants overaccumulated *MIR395* which targets several ATP sulfurylases (APS1, APS3, and APS4), leading to a drastic reduction of *APS1* transcripts (Jones-Rhoades and Bartel, 2004). Later on, several other miRNAs or siRNAs were shown to be regulated by abiotic stresses (cold, drought, and salt stresses) or ABA treatments (Sunkar and Zhu, 2004). For example, *MIR399* plays a key role in P homeostasis in Arabidopsis (Bari *et al.*, 2006; Chiou *et al.*, 2006; Fujii *et al.*, 2005).

MIR399 is strongly induced during P starvation, whereas the expression of one target transcript, *UBC* (for ubiquitin-conjugating enzyme), is concomitantly reduced. In contrast to the majority of plant miRNAs, *MIR399* does not bind a single site in the transcript-coding region but several recognition sites present in the 5′UTR. Plants expressing an *MIR399*-resistant *UBC* transcript show a reduced response of the primary root to low P concentrations. In addition, *MIR399* overexpression leads to the disappearance of endogenous *UBC* transcripts and increased P accumulation in the plant. This demonstrates that the *MIR399-UBC* pair plays a key role in the control of P homeostasis in Arabidopsis. Another well-studied example is MIR398 that regulates mRNAs encoding a cytosolic (CSD1) or a chloroplastic (CSD2) form of a Cu/Zn superoxide dismutase (Sunkar *et al.*, 2006). During an oxidative stress, *MIR398* expression is reduced, whereas its target transcripts accumulate. This response likely allows plant cells to cope with ROS. Plants expressing an *MIR398*-resistant CSD2 mRNA were more tolerant to an oxidative stress demonstrating the major role of CSD2 and its MIR398-mediated regulation in plant stress responses. Considering that S and P deprivations through ROS action have major consequences in root architecture (see Section IV), we can speculate that miRNA-mediated regulation could participate in root responses to these stresses.

A new mechanism involving siRNAs in stress responses has been recently discovered in Arabidopsis (Borsani *et al.*, 2005). Under salt stress conditions, a 24-nt-long siRNA could be detected, coming from two partially overlapping mRNAs that are in antisense configuration. A dsRNA (around 700 bp) is formed by complementarity between a constitutively expressed gene encoding a pyrroline-5-carboxylate dehydrogenase (P5CDH; involved in proline homeostasis) and an antisense stress-inducible transcript, *SRO5*, of unknown function. This dsRNA is processed into so-called nat-siRNAs. The latter induces the cleavage of *P5CDH* transcripts, acting thus as true siRNAs and leading to a complete extinction of this gene under stress conditions. This *SRO5*-mediated downregulation of P5CDH allows the accumulation of proline, an osmolyte known to be involved in stress responses. In Arabidopsis, the actual estimates of overlapping genes (potential antisense RNAs) being around 2000, such nat-siRNA-mediated regulation could have a strong impact on a variety of conditions including stress responses, hormone signaling, and differentiation processes. Nevertheless, other examples of nat-siRNAs are needed to further support this particular regulation pathway.

Regulatory RNAs not only affect abiotic responses but are also involved in biotic interactions. During the compatible interaction between the pathogen *P. syringae* and Arabidopsis, an miRNA seems to participate in plant defense

responses. Transcripts coding for particular category of auxin receptors (TIR1, AFB2, and AFB3) are negatively regulated by *MIR393*, which is induced by the bacterial elicitor flagellin. Since several pathogens produce auxin and this hormone may intervene in the infection process, the MIR393-mediated repression of hormone receptors may be linked to a natural "immune" response of the plant to control pathogen infection (Navarro *et al.*, 2006). Knowing the major role of auxin in root development, MIR393 could also be involved in pathogen responses in roots. Other biotic interactions are beneficial for the plant as the mentioned symbiotic interaction between *Rhizobium* and legume plants. In *M. truncatula*, an HAP transcription factor has been shown to be essential for nodule differentiation and the corresponding mRNA is spatially controlled by MIR169 (Combier *et al.*, 2006). Abolishment of this posttranscriptional regulation (using an MIR-resistant version of the *MtHAP2–1* mRNA) leads to delayed nodule development, likely due to misregulated meristematic activity.

Due to the large diversity of these novel regulatory RNAs, we are only beginning to identify a wide variety of processes that may be controlled posttranscriptionally (Lu *et al.*, 2005; Rajagopalan *et al.*, 2006). Potential roles of miRNAs in root development or responses to abiotic stresses are summarized in Fig. 3.

VI. CONCLUDING REMARKS

In contrast to animals, plants adapt to the environment by modulating their growth and differentiation. The meristematic cells integrate signals from the external conditions to regulate specific developmental responses and cope with environmental constraints. Both postembryonic development and response to environmental conditions require the activation of hormone-related signaling pathways. The appropriate developmental response to a given stress is therefore the result of the integration of many signals perceived by the plant and their cross talk with hormone action. Analyses are even more complicated when plants overcome a stress due to inorganic nutrient deficiencies such as phosphate, nitrate, and sulfate. These nutrients and/or their metabolites can act as signal molecules directly affecting plant development or through interactions with hormonal signaling pathways. QTL approaches are likely to be very useful in the dissection of such pathways. Moreover, experimental procedures (e.g., culture conditions, nutrient concentrations) are variables between studies aiming to describe the same phenomenon, namely a nutrient deficiency or excess. One can thus only infer tendencies from the synthesis of the actual data. As it is already done for the

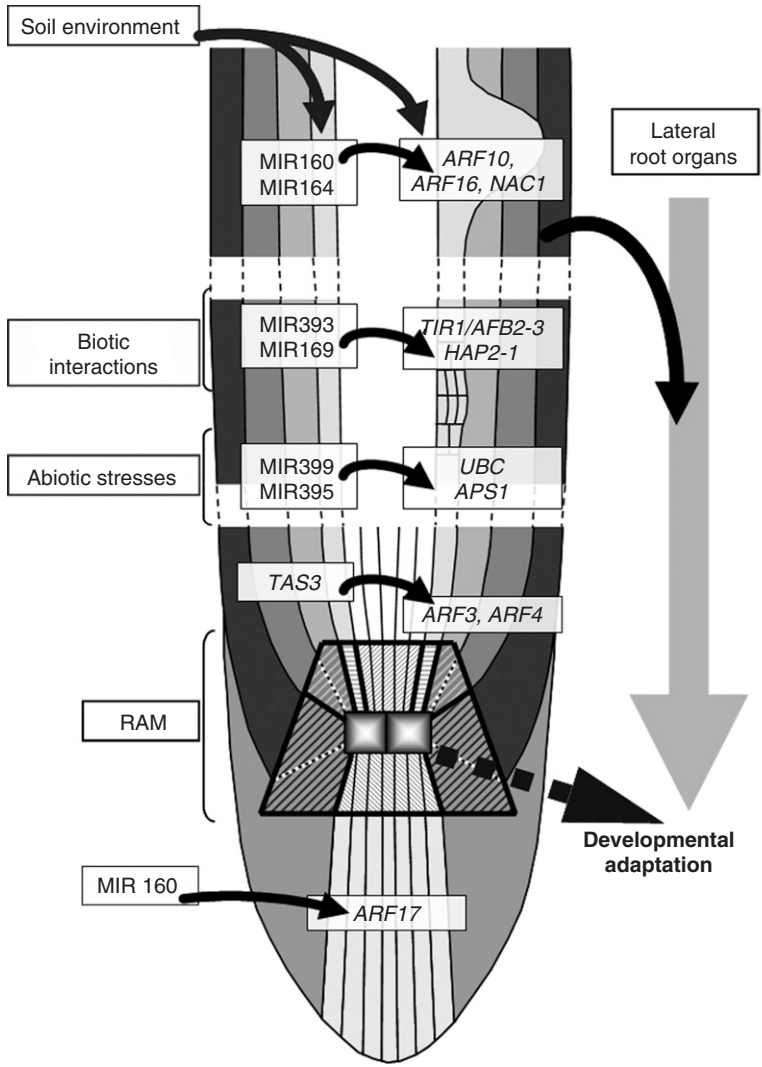

Fig. 3. RNA-mediated regulation of root architecture. Integration of riboregulation with environmental and endogenous signaling pathways. RAM, root apical meristem; gene names are mentioned in the text.

homologation of new crop cultivars or expression profiling via DNA arrays, meta-analyses studies (e.g., comparing data from different experimental conditions in different laboratories using many mutants and/or genotypes) should be launched to define in an unambiguous manner the phenotypes linked to environmental modifications. The results obtained by Bray (2004)

from the comparison of transcriptomic data on water stress experiments in Arabidopsis revealed the importance to analyze the overlap existing between different studies. This may help to discriminate between specific targets and ''noise'' variation due to the environment in transcriptional profiles. These analyses need to be reinforced in the future so that large-scale data obtained on model plants (as Arabidopsis or *M. truncatula*) can be translated in useful agronomic traits for crops.

In addition to the diverse mechanisms implied in the regulation of root growth, which involve homeostasis and signaling pathways of several hormones, posttranscriptional regulation of developmental regulators mediated by noncoding RNAs is emerging as an important determinant of differentiation in eukaryotes. These novel regulatory mechanisms may be particularly relevant to adjust differentiation processes to the environmental conditions encountered during growth. In roots, developmental plasticity accounts mainly for the adaptation of root architecture to the soil conditions (involving parameters such as water and mineral levels or interactions with symbiotic microorganisms). Environmental responses may be integrated in the root system through the action of specific regulators, such as transcription factors, on primary root and LR developmental programs. As mRNAs encoding transcription factors seem privileged targets of miRNAs, temporal and spatial regulation of miRNA-target transcription factor interactions may play significant roles in the adaptation of root architecture to the soil environment.

ACKNOWLEDGMENTS

M.J. and P.L. are supported by the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche (MENR). S.G.R. was the recipient of a grant from Consejo Nacional de Ciencia y Tecnologia, Mexico. The support of the ''Grain legume'' and RIBOREG FP6 EEC project are also acknowledged.

REFERENCES

Abel, S., Oeller, P. W. and Theologis, A. (1994). Early auxin-induced genes encode short-lived nuclear proteins. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 326–330.

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J. and Harberd, N. P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. *Science* **311**, 91–94.

Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y. S., Amasino, R. and Scheres, B. (2004). The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. *Cell* **119**, 109–120.

Aloni, R., Aloni, E., Langhans, M. and Ullrich, C. I. (2006). Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Annals of Botany (London)* **97**, 883–893.

Arrese-Igor, C., González, E. M., Gordon, A. J., Minchin, F. R., Gálvez, L., Royuela, M., Cabrerizo, P. M. and Aparicio-Tejo, P. M. (1999). Sucrose synthase and nodule nitrogen fixation under drought and other environmental stresses. *Symbiosis* **27**, 189–212.

Asami, T., Sun-Young, H., Kitahata, N., Saito, T., Kobayashi, M., Shinozaki, K., Nakano, T. and Nakashima, K. (2003). Drug design of abscisic acid biosynthesis inhibitor and its effect on plants. *Plant and Cell Physiology* **45**, 154.

Axtell, M. J., Jan, C., Rajagopalan, R. and Bartel, D. P. (2006). A two-hit trigger for siRNA biogenesis in plants. *Cell* **127**, 565–577.

Bari, R., Datt Pant, B., Stitt, M. and Scheible, W. R. (2006). PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiology* **141**, 988–999.

Bates, T. R. and Lynch, J. P. (2000). Plant growth and phosphorus accumulation of wild type and two root hair mutants of *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* **87**, 958–963.

Beaudoin, N., Serizet, C., Gosti, F. and Giraudat, J. (2000). Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* **12**, 1103–1115.

Beeckman, T., Burssens, S. and Inze, D. (2001). The peri-cell-cycle in Arabidopsis. *Journal of Experimental Botany* **52**, 403–411.

Benfey, P. N. and Scheres, B. (2000). Root development. *Current Biology* **10**, R813–R815.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G. and Friml, J. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* **115**, 591–602.

Bennett, M. J., Marchant, A., Green, H. G., May, S. T., Ward, S. P., Millner, P. A., Walker, A. R., Schulz, B. and Feldmann, K. A. (1996). Arabidopsis AUX1 gene: A permease-like regulator of root gravitropism. *Science* **273**, 948–950.

Berger, F., Haseloff, J., Schiefelbein, J. and Dolan, L. (1998). Positional information in root epidermis is defined during embryogenesis and acts in domains with strict boundaries. *Current Biology* **8**, 421–430.

Berleth, T. and Jürgens, G. (1993). The role of the *monopteros* gene in organising the basal body region of the Arabidopsis embryo. *Development* **118**, 575–587.

Bernhardt, C., Lee, M. M., Gonzalez, A., Zhang, F., Lloyd, A. and Schiefelbein, J. (2003). The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. *Development* **130**, 6431–6439.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. and Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* **433**, 39–44.

Boerjan, W., Cervera, M. T., Delarue, M., Beeckman, T., Dewitte, W., Bellini, C., Caboche, M., Van Onckelen, H., Van Montagu, M. and Inze, D. (1995). Superroot, a recessive mutation in Arabidopsis, confers auxin overproduction. *Plant Cell* **7**, 1405–1419.

Bollman, K. M., Aukerman, M. J., Park, M. Y., Hunter, C., Berardini, T. Z. and Poethig, R. S. (2003). HASTY, the Arabidopsis ortholog of exportin 5/MSN5, regulates phase change and morphogenesis. *Development* **130**, 1493–1504.

Borsani, O., Zhu, J., Verslues, P. E., Sunkar, R. and Zhu, J. K. (2005). Endogenous siRNAs derived from a pair of natural cis-antisense transcripts regulate salt tolerance in Arabidopsis. *Cell* **123**, 1279–1291.

Bouche, N., Lauressergues, D., Gasciolli, V. and Vaucheret, H. (2006). An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. *The EMBO Journal* **25**, 3347–3356.

Brachmann, A. (2006). Plant-fungal symbiosis en gros and en detail. *The New Phytologist* **171**, 242–246.

Brady, S. M., Sarkar, S. F., Bonetta, D. and McCourt, P. (2003). The ABSCISIC ACID INSENSITIVE 3 (ABI3) gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in Arabidopsis. *The Plant Journal* **34**, 67–75.

Bray, E. A. (2004). Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*. *Journal of Experimental Botany* **55**, 2331–2341.

Brewin, N. J. (1991). Development of the legume root nodule. *Annual Review of Cell Biology* **7**, 191–226.

Bright, L. J., Liang, Y., Mitchell, D. M. and Harris, J. M. (2005). The LATD gene of *Medicago truncatula* is required for both nodule and root development. *Molecular Plant Microbe Interactions* **18**, 521–532.

Caetano-Anollés, G. and Gresshoff, P. M. (1991). Plant genetic control of nodulation. *Annual Review of Microbiology* **45**, 345–382.

Carroll, B. J., McNeil, D. L. and Gresshoff, P. M. (1985). A supernodulation and nitrate-tolerant symbiotic (nts) soybean mutant. *Plant Physiology* **78**, 34–40.

Casimiro, I., Beeckman, T., Graham, N., Bhalerao, R., Zhang, H., Casero, P., Sandberg, G. and Bennett, M. J. (2003). Dissecting Arabidopsis lateral root development. *Trends in Plant Science* **8**, 165–171.

Celenza, J. L., Jr., Grisafi, P. L. and Fink, G. R. (1995). A pathway for lateral root formation in *Arabidopsis thaliana*. *Genes & Development* **9**, 2131–2142.

Chen, J. G., Ullah, H., Temple, B., Liang, J., Guo, J., Alonso, J. M., Ecker, J. R. and Jones, A. M. (2006). RACK1 mediates multiple hormone responsiveness and developmental processes in Arabidopsis. *Journal of Experimental Botany* **57**, 2697–2708.

Chen, X., Liu, J., Cheng, Y. and Jia, D. (2002). HEN1 functions pleiotropically in Arabidopsis development and acts in C function in the flower. *Development* **129**, 1085–1094.

Chilley, P. M., Casson, S. A., Tarkowski, P., Hawkins, N., Wang, K. L., Hussey, P. J., Beale, M., Ecker, J. R., Sandberg, G. K. and Lindsey, K. (2006). The POLARIS peptide of Arabidopsis regulates auxin transport and root growth via effects on ethylene signaling. *Plant Cell* **18**, 3058–3072.

Chiou, T. J., Aung, K., Lin, S. I., Wu, C. C., Chiang, S. F. and Su, C. L. (2006). Regulation of phosphate homeostasis by MicroRNA in Arabidopsis. *Plant Cell* **18**, 412–421.

Combier, J. P., Frugier, F., de Billy, F., Boualem, A., El-Yahyaoui, F., Moreau, S., Vernie, T., Ott, T., Gamas, P., Crespi, M. and Niebel, A. (2006). MtHAP2–1 is a key transcriptional regulator of symbiotic nodule development regulated by microRNA169 in *Medicago truncatula*. *Genes & Development* **20**, 3084–3088.

Costa, S. and Shaw, P. (2006). Chromatin organization and cell fate switch respond to positional information in Arabidopsis. *Nature* **439**, 493–496.

Crespi, M. and Galvez, S. (2000). Molecular mechanisms in root nodule development. *Journal of Plant Growth Regulation* **19**, 155–166.

Dan, H., Yang, G. and Zheng, Z. L. (2007). A negative regulatory role for auxin in sulphate deficiency response in *Arabidopsis thaliana*. *Plant Molecular Biology* **63**, 221–235.

Day, D. A., Lambers, H., Bateman, J., Carroll, B. J. and Gresshoff, P. M. (1986). Growth comparisons of a supernodulating soybean (*Glycine max*) mutant and its wild-type parent. *Physiologia Plantarum* **68**, 375–382.

Dazzo, F. B. and Brill, W. J. (1978). Regulation by fixed nitrogen of host-symbiont recognition in the rhizobium-clover symbiosis. *Plant Physiology* **62**, 18–21.

De Smet, I., Signora, L., Beeckman, T., Inze, D., Foyer, C. H. and Zhang, H. (2003). An abscisic acid-sensitive checkpoint in lateral root development of Arabidopsis. *The Plant Journal* **33**, 543–555.

De Smet, I., Vanneste, S., Inze, D. and Beeckman, T. (2006). Lateral root initiation or the birth of a new meristem. *Plant Molecular Biology* **60**, 871–887.

De Smet, I., Tetsumura, T., De Rybel, B., Frey, N. F., Laplaze, L., Casimiro, I., Swarup, R., Naudts, M., Vanneste, S., Audenaert, D., Inze, D., Bennett, M. J., *et al.* (2007). Auxin-dependent regulation of lateral root positioning in the basal meristem of Arabidopsis. *Development* **134**, 681–690.

Deak, K. I. and Malamy, J. (2005). Osmotic regulation of root system architecture. *The Plant Journal* **43**, 17–28.

del Papa, M. F., Balague, L. J., Sowinski, S. C., Wegener, C., Segundo, E., Abarca, F. M., Toro, N., Niehaus, K., P hler, A., Aguilar,O. M., Martinez-Drets, G. and Lagares, A. (1999). Isolation and characterization of alfalfa-nodulating rhizobia present in acidic soils of central argentina and uruguay. *Applied and Environmental Microbiology* **65**, 1420–1427.

Dharmasiri, N., Dharmasiri, S. and Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. *Nature* **435**, 441–445.

Di Laurenzio, L., Wysocka-Diller, J., Malamy, J. E., Pysh, L., Helariutta, Y., Freshour, G., Hahn, M. G., Feldmann, K. A. and Benfey, P. N. (1996). The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the Arabidopsis root. *Cell* **86**, 423–433.

DiDonato, R. J., Arbuckle, E., Buker, S., Sheets, J., Tobar, J., Totong, R., Grisafi, P., Fink, G. R. and Celenza, J. L. (2004). Arabidopsis ALF4 encodes a nuclear-localized protein required for lateral root formation. *The Plant Journal* **37**, 340–353.

Dolan, L. and Costa, S. (2001). Evolution and genetics of root hair stripes in the root epidermis. *Journal of Experimental Botany* **52**, 413–417.

Dubrovsky, J. G., Doerner, P. W., Colon-Carmona, A. and Rost, T. L. (2000). Pericycle cell proliferation and lateral root initiation in Arabidopsis. *Plant Physiology* **124**, 1648–1657.

Dubrovsky, J. G., Gambetta, G. A., Hernandez-Barrera, A., Shishkova, S. and Gonzalez, I. (2006). Lateral root initiation in Arabidopsis: Developmental window, spatial patterning, density and predictability. *Annals of Botany (London)* **97**, 903–915.

Duzan, H. M., Zhou, X., Souleimanov, A. and Smith, D. L. (2004). Perception of *Bradyrhizobium japonicum* nod factor by soybean [*Glycine max* (L.) Merr.] root hairs under abiotic stress conditions. *Journal of Experimental Botany* **55**, 2641–2646.

Fahlgren, N., Montgomery, T. A., Howell, M. D., Allen, E., Dvorak, S. K., Alexander, A. L. and Carrington, J. C. (2006). Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. *Current Biology* **16**, 939–944.

Feldman, L. (1994). The maize roots. *In* "The Maize Handbook" (M. Freeling and V. Walbot, eds.), pp. 29–37. Springer, New York.

Ferreira, F. J. and Kieber, J. J. (2005). Cytokinin signaling. *Current Opinion in Plant Biology* **8**, 518–525.

Fitz Gerald, J. N., Lehti-Shiu, M. D., Ingram, P. A., Deak, K. I., Biesiada, T. and Malamy, J. E. (2006). Identification of quantitative trait loci that regulate Arabidopsis root system size and plasticity. *Genetics* **172**, 485–498.

Foreman, J., Demidchik, V., Bothwell, J. H., Mylona, P., Miedema, H., Torres, M. A., Linstead, P., Costa, S., Brownlee, C., Jones, J. D., Davies, J. M. and Dolan, L. (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442–446.

Franco-Zorrilla, J. M., Martin, A. C., Solano, R., Rubio, V., Leyva, A. and Paz-Ares, J. (2002). Mutations at CRE1 impair cytokinin-induced repression of phosphate starvation responses in Arabidopsis. *The Plant Journal* **32**, 353–360.

Franco-Zorrilla, J. M., Martin, A. C., Leyva, A. and Paz-Ares, J. (2005). Interaction between phosphate-starvation, sugar, and cytokinin signaling in Arabidopsis and the roles of cytokinin receptors CRE1/AHK4 and AHK3. *Plant Physiology* **138**, 847–857.

Friml, J., Benfey, P., Benkova, E., Bennett, M., Berleth, T., Geldner, N., Grebe, M., Heisler, M., Hejatko, J., Jurgens, G., Laux, T., Lindsey, K., *et al*., (2006). Apical-basal polarity: Why plant cells don't stand on their heads. *Trends in Plant Science* **11**, 12–14.

Frugier, F., Kondorosi, A. and Crespi, M. (1998). Identification of novel putative regulatory genes induced during alfalfa nodule development with a cold-plaque screening procedure. *Moleculor Plant Microbe Interactions* **11**, 358–366.

Frugier, F., Poirier, S., Satiat-Jeunemaitre, B., Kondorosi, A. and Crespi, M. (2000). A Kruppel-like zinc finger protein is involved in nitrogen-fixing root nodule organogenesis. *Genes & Development* **14**, 475–482.

Fu, X. and Harberd, N. P. (2003). Auxin promotes Arabidopsis root growth by modulating gibberellin response. *Nature* **421**, 740–743.

Fujii, H., Chiou, T. J., Lin, S. I., Aung, K. and Zhu, J. K. (2005). A miRNA involved in phosphate-starvation response in Arabidopsis. *Current Biology* **15**, 2038–2043.

Fukaki, H., Tameda, S., Masuda, H. and Tasaka, M. (2002). Lateral root formation is blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. *The Plant Journal* **29**, 153–168.

Fukaki, H., Nakao, Y., Okushima, Y., Theologis, A. and Tasaka, M. (2005). Tissue-specific expression of stabilized SOLITARY-ROOT/IAA14 alters lateral root development in Arabidopsis. *The Plant Journal* **44**, 382–395.

Gage, D. J. (2004). Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate legumes. *Microbiology and Molecular Biology Reviews* **68**, 280–300.

Gallagher, K. L., Paquette, A. J., Nakajima, K. and Benfey, P. N. (2004). Mechanisms regulating SHORT-ROOT intercellular movement. *Current Biology* **14**, 1847–1851.

Gan, Y., Filleur, S., Rahman, A., Gotensparre, S. and Forde, B. G. (2005). Nutritional regulation of ANR1 and other root-expressed MADS-box genes in *Arabidopsis thaliana*. *Planta* **222**, 730–742.

Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I. and Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *Bioessays* **28**, 1091–1101.

Geurts, R., Fedorova, E. and Bisseling, T. (2005). Nod factor signaling genes and their function in the early stages of Rhizobium infection. *Current Opinion in Plant Biology* **8**, 346–352.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y. and McCourt, P. (2000). Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. *Plant Cell* **12**, 1117–1126.

Gianinazzi-Pearson, V., Séjalon-Delmas, N., Genre, A., Jeandroz, S. and Bonfante, P. (2007). Plants and arbuscular mycorrhizal fungi: Cues and communication in the early steps of symbiotic interactions. *Advances in Botanical Research* **46**, 181–207.

Goda, H., Sawa, S., Asami, T., Fujioka, S., Shimada, Y. and Yoshida, S. (2004). Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. *Plant Physiology* **134**, 1555–1573.

Graham, J. H. and Miller, R. M. (2005). Mycorrhizas: Gene to function. *Plant and Soil* **274**, 79–100.

Gresshoff, P. M. (1993). Molecular genetic analysis of nodulation genes in soybean. *Plant Breeding Reviews* **11**, 275–318.

Guo, H. S., Xie, Q., Fei, J. F. and Chua, N. H. (2005). MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for Arabidopsis lateral root development. *Plant Cell* **17**, 1376–1386.

Hamilton, A. J. and Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**, 950–952.

Hamman, T., Benkova, E., Bäurle, I., Kientz, M. and Jürgens, G. (2002). The Arabidopsis *BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediating embryo patterning. *Genes & Development* **16**, 1610–1615.

Han, K. H., Bradshaw, H. and Gordon, M. (1994). Adventitious root and shoot regeneration *in vitro* is under major gene control in an F2 family of hybrid poplar (*Populus trichocarpa* × *P. deltoides*). *For Genet* **1**, 139–146.

Harrison, M. J. (2005). Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* **59**, 19–42.

Hasegawa, P. M. (2000). Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463–499.

Helariutta, Y., Fukaki, H., Wysocka-Diller, J., Nakajima, K., Jung, J., Sena, G., Hauser, M. T. and Benfey, P. N. (2000). The SHORT-ROOT gene controls radial patterning of the Arabidopsis root through radial signaling. *Cell* **101**, 555–567.

Higuchi, M., Pischke, M. S., Mahonen, A. P., Miyawaki, K., Hashimoto, Y., Seki, M., Kobayashi, M., Shinozaki, K., Kato, T., Tabata, S., Helariutta, Y., Sussman, M. R., *et al.* (2004). In planta functions of the Arabidopsis cytokinin receptor family. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 8821–8826.

Hirsch, A. M. (1992). Developmental biology of legume nodulation. *The New Phytologist* **122**, 211–237.

Hirsch, A. M. and LaRue, T. A. (1997). Is the legume nodule a modified root or stem or an organ sui generis? *Critical Reviews in Plant Sciences* **16**, 361–392.

Horgan, J. M. and Wareing, P. F. (1980). Cytokinin and the growth responses of seedlings of *Betula pendula* to nitrogen and phosphorus deficiency. *Journal of Experimental Botany* **31**, 525–532.

Jacobsen, S. E., Running, M. P. and Meyerowitz, E. M. (1999). Disruption of an RNA helicase/RNAse III gene in Arabidopsis causes unregulated cell division in floral meristems. *Development* **126**, 5231–5243.

Jones-Rhoades, M. W. and Bartel, D. P. (2004). Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. *Molecular Cell* **14**, 787–799.

Jost, R., Altschmied, L., Bloem, E., Bogs, J., Gershenzon, J., Hahnel, U., Hansch, R., Hartmann, T., Kopriva, S., Kruse, C., Mendel, R. R., Papenbrock, J., *et al.* (2005). Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulfur-related pathways in *Arabidopsis thaliana*. *Photosynthesis Research* **86**, 491–508.

Kepinski, S. and Leyser, O. (2005). The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature* **435**, 446–451.

Kidner, C. A. and Martienssen, R. A. (2005). The role of ARGONAUTE1 (AGO1) in meristem formation and identity. *Developmental Biology* **280**, 504–517.

King, J. J. and Stimart, D. P. (1998). Genetic analysis of variation for auxin-induced adventitious root formation among eighteen ecotypes of *Arabidopsis thaliana* L. Heynh. *The Journal of Heredity* **89**, 481–487.

Kopriva, S. and Rennenberg, H. (2004). Control of sulphate assimilation and glutathione synthesis: Interaction with N and C metabolism. *Journal of Experimental Botany* **55**, 1831–1842.

Krusell, L., Madsen, L. H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N., *et al.* (2002). Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature* **420**, 422–426.

Kurihara, Y. and Watanabe, Y. (2004). Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 12753–12758.

Kutz, A., Muller, A., Hennig, P., Kaiser, W. M., Piotrowski, M. and Weiler, E. W. (2002). A role for nitrilase 3 in the regulation of root morphology in sulphur-starving *Arabidopsis thaliana*. *The Plant Journal* **30**, 95–106.

Lagos-Quintana, M., Rauhut, R., Lendeckel, W. and Tuschl, T. (2001). Identification of novel genes coding for small expressed RNAs. *Science* **294**, 853–858.

Lanter, D. J., Munns, D. N. and Clarkin, K. L. (1981). Salt response of chickpeas influenced by N supply. *Agronomy Journal* **96**, 1–966.

Lee, R. C., Feinbaum, R. L. and Ambros, V. (1993). The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75**, 843–854.

Levesque, M. P., Vernoux, T., Busch, W., Cui, H., Wang, J. Y., Blilou, I., Hassan, H., Nakajima, K., Matsumoto, N., Lohmann, J. U., Scheres, B. and Benfey, P. N. (2006). Whole-genome analysis of the SHORT-ROOT developmental pathway in Arabidopsis. *PLoS Biology* **4**, e143.

Li, M., Qin, C., Welti, R. and Wang, X. (2006). Double knockouts of phospholipases Dzeta1 and Dzeta2 in Arabidopsis affect root elongation during phosphate-limited growth but do not affect root hair patterning. *Plant Physiology* **140**, 761–770.

Liang, Y., Mitchell, D. M. and Harris, J. M. (2007). Abscissic acid rescues the root meristem defects of the *Medicago truncatula latd* mutant. *Developmental Biology* **304**, 297–307.

Linkohr, B. I., Williamson, L. C., Fitter, A. H. and Leyser, H. M. (2002). Nitrate and phosphate availability and distribution have different effects on root system architecture of Arabidopsis. *The Plant Journal* **29**, 751–760.

Little, D. Y., Rao, H., Oliva, S., Daniel-Vedele, F., Krapp, A. and Malamy, J. E. (2005). The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 13693–13698.

Ljung, K., Hull, A. K., Celenza, J., Yamada, M., Estelle, M., Normanly, J. and Sandberg, G. (2005). Sites and regulation of auxin biosynthesis in Arabidopsis roots. *Plant Cell* **17**, 1090–1104.

Lopez-Bucio, J., Hernandez-Abreu, E., Sanchez-Calderon, L., Nieto-Jacobo, M. F., Simpson, J. and Herrera-Estrella, L. (2002). Phosphate availability alters architecture and causes changes in hormone sensitivity in the Arabidopsis root system. *Plant Physiology* **129**, 244–256.

Lopez-Bucio, J., Cruz-Ramirez, A. and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Current Opinion in Plant Biology* **6**, 280–287.

Loudet, O., Gaudon, V., Trubuil, A. and Daniel-Vedele, F. (2005). Quantitative trait loci controlling root growth and architecture in *Arabidopsis thaliana* confirmed by heterogeneous inbred family. *Theoretical and Applied Genetics* **110**, 742–753.

Lu, C., Tej, S. S., Luo, S., Haudenschild, C. D., Meyers, B. C. and Green, P. J. (2005). Elucidation of the small RNA component of the transcriptome. *Science* **309**, 1567–1569.

Lynch, J. (1995). Root architecture and plant productivity. *Plant Physiology* **109**, 7–13.

Ma, Z., Bielenberg, D. G., Brown, K. M. and Lynch, J. P. (2001). Regulation of root hair density by phosphorus availability in *Arabidopsis thaliana*. *Plant, Cell & Environment* **24**, 459–467.

Malamy, J. E. (2005). Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment* **28**, 67–77.

Malamy, J. E. and Benfey, P. N. (1997a). Down and out in Arabidopsis: The formation of lateral roots. *Trends in plant science* **2**, 390–396.

Malamy, J. E. and Benfey, P. N. (1997b). Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. *Development* **124**, 33–44.

Malamy, J. E. and Ryan, K. S. (2001). Environmental regulation of lateral root initiation in Arabidopsis. *Plant Physiology* **127**, 899–909.

Mallory, A. C. and Vaucheret, H. (2006). Functions of microRNAs and related small RNAs in plants. *Nat Genet* **38**(Suppl.), S31–S36.

Mallory, A. C., Bartel, D. P. and Bartel, B. (2005). MicroRNA-directed regulation of Arabidopsis AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. *Plant Cell* **17**, 1360–1375.

Mallory, T. E., Chiang, S.-H., Cutter, E. G. and Gifford, E. M. (1970). Sequence and pattern of lateral root formation in five selected species. *American Journal of Botany* **57**, 800–809.

Marchant, A., Bhalerao, R., Casimiro, I., Eklof, J., Casero, P. J., Bennett, M. and Sandberg, G. (2002). AUX1 promotes lateral root formation by facilitating

indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *Plant Cell* **14**, 589–597.

Marques, C., Vasquez-Kool, J., Carocha, V., Ferreira, J., O'Malley, D., Liu, B. and Sederoff, R. (1999). Genetic dissection of vegetative traits in *Eucalyptus tereticornis* and *E. globulus*. *Theoretical and Applied Genetics* **99**, 936–946.

Martin, A. C., del Pozo, J. C., Iglesias, J., Rubio, V., Solano, R., de La Pena, A., Leyva, A. and Paz-Ares, J. (2000). Influence of cytokinins on the expression of phosphate starvation responsive genes in Arabidopsis. *The Plant Journal* **24**, 559–567.

Maruyama-Nakashita, A., Nakamura, Y., Yamaya, T. and Takahashi, H. (2004). Regulation of high-affinity sulphate transporters in plants: Towards systematic analysis of sulphur signalling and regulation. *Journal of Experimenal Botany* **55**, 1843–1849.

Maruyama-Nakashita, A., Nakamura, Y., Watanabe-Takahashi, A., Inoue, E., Yamaya, T. and Takahashi, H. (2005). Identification of a novel cis-acting element conferring sulfur deficiency response in Arabidopsis roots. *The Plant Journal* **42**, 305–314.

Mason, M. G., Li, J., Mathews, D. E., Kieber, J. J. and Schaller, G. E. (2004). Type-B response regulators display overlapping expression patterns in Arabidopsis. *Plant Physiology* **135**, 927–937.

Mason, M. G., Mathews, D. E., Argyros, D. A., Maxwell, B. B., Kieber, J. J., Alonso, J. M., Ecker, J. R. and Schaller, G. E. (2005). Multiple type-B response regulators mediate cytokinin signal transduction in Arabidopsis. *Plant Cell* **17**, 3007–3018.

Mathesius, U., Weinman, J. J., Rolfe, B. G. and Djordjevic, M. A. (2000). Rhizobia can induce nodules in white clover by "hijacking" mature cortical cells activated during lateral root development. *Moleculor Plant Microbe Interactions* **13**, 170–182.

Merchan, F., Breda, C., Perez Hormaeche, J., Sousa, C., Kondorosi, A., Aguilar, O. M., Megias, M. and Crespi, M. (2003). A Krüppel-like transcription factor gene is involved in salt stress responses in Medicago spp. *Plant and Soil* **257**, 1–9.

Miller, C. R., Ochoa, I., Nielsen, K. L., Beck, D. and Lynch, J. P. (2003). Genetic variation for adventitious rooting in response to low phosphors availability: Potential utility for phosphorus acquisition from stratified soils. *Functional Plant Biology* **30**, 973–985.

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**, 15–19.

Miura, K., Rus, A., Sharkhuu, A., Yokoi, S., Karthikeyan, A. S., Raghothama, K. G., Baek, D., Koo, Y. D., Jin, J. B., Bressan, R. A., Yun, D. J. and Hasegawa, P. M. (2005). The Arabidopsis SUMO E3 ligase SIZ1 controls phosphate deficiency responses. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 7760–7765.

Miyawaki, K., Matsumoto-Kitano, M. and Kakimoto, T. (2004). Expression of cytokinin biosynthetic isopentenyltransferase genes in Arabidopsis: Tissue specificity and regulation by auxin, cytokinin, and nitrate. *The Plant Journal* **37**, 128–138.

Molina-Farero, C., Creus, C. M., Lanteri, M. L., Correa-Aragunde, N., Lombardo, M. C., Barassi, C. A. and Lamattina, L. (2007). Nitric oxide and plant growth promoting rhizobacteria: Common features influencing root growth and development. *Advances in Botanical Research* **46**, 1–23.

Mouchel, C. F., Briggs, G. C. and Hardtke, C. S. (2004). Natural genetic variation in Arabidopsis identifies BREVIS RADIX, a novel regulator of cell proliferation and elongation in the root. *Genes & Development* **18**, 700–714.

Mouchel, C. F., Osmont, K. S. and Hardtke, C. S. (2006). BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* **443**, 458–461.

Nacry, P., Canivenc, G., Muller, B., Azmi, A., Van Onckelen, H., Rossignol, M. and Doumas, P. (2005). A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in Arabidopsis. *Plant Physiology* **138**, 2061–2074.

Nag, R., Maity, M. K. and Dasgupta, M. (2005). Dual DNA binding property of ABA insensitive 3 like factors targeted to promoters responsive to ABA and auxin. *Plant Molecular Biology* **59**, 821–838.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J. D. (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science* **312**, 436–439.

Nawy, T., Lee, J. Y., Colinas, J., Wang, J. Y., Thongrod, S. C., Malamy, J. E., Birnbaum, K. and Benfey, P. N. (2005). Transcriptional profile of the Arabidopsis root quiescent center. *Plant Cell* **17**, 1908–1925.

Nemhauser, J. L. and Chory, J. (2004). BRing it on: New insights into the mechanism of brassinosteroid action. *Journal Experimental Botany* **55**, 265–270.

Nemhauser, J. L., Mockler, T. C. and Chory, J. (2004). Interdependency of brassinosteroid and auxin signaling in Arabidopsis. *PLoS Biology* **2**, E258.

Nikiforova, V., Freitag, J., Kempa, S., Adamik, M., Hesse, H. and Hoefgen, R. (2003). Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: Interlacing of biosynthetic pathways provides response specificity. *The Plant Journal* **33**, 633–650.

Nikiforova, V. J., Daub, C. O., Hesse, H., Willmitzer, L. and Hoefgen, R. (2005). Integrative gene-metabolite network with implemented causality deciphers informational fluxes of sulphur stress response. *Journal Experimental Botany* **56**, 1887–1896.

Nishimura, C., Ohashi, Y., Sato, S., Kato, T., Tabata, S. and Ueguchi, C. (2004). Histidine kinase homologs that act as cytokinin receptors possess overlapping functions in the regulation of shoot and root growth in Arabidopsis. *Plant Cell* **16**, 1365–1377.

Nishimura, R., Ohmori, M. and Kawaguchi, M. (2002). The novel symbiotic phenotype of enhanced-nodulating mutant of *Lotus japonicus*: Astray mutant is an early nodulating mutant with wider nodulation zone. *Plant Cell Physiology* **43**, 853–859.

Ohashi, Y., Oka, A., Rodrigues-Pousada, R., Possenti, M., Ruberti, I., Morelli, G. and Aoyama, T. (2003). Modulation of phospholipid signaling by GLABRA2 in root-hair pattern formation. *Science* **300**, 1427–1430.

Okushima, Y., Fukaki, H., Onoda, M., Theologis, A. and Tasaka, M. (2007). ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in Arabidopsis. *Plant Cell* **19**, 118–130.

Oldroyd, G. E. and Downie, J. A. (2004). Calcium, kinases and nodulation signalling in legumes. *Nature Reviews Molecular Cell Biology* **5**, 566–576.

Pasquinelli, A. E., Reinhart, B. J., Slack, F., Martindale, M. Q., Kuroda, M. I., Maller, B., Hayward, D. C., Ball, E. E., Degnan, B., Muller, P., Spring, J., Srinivasan, A., *et al.* (2000). Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA. *Nature* **408**, 86–89.

Penmetsa, R. V. and Cook, D. R. (1997). A legume ethylene-insensitive mutant hyperinfected by its rhizobial symbiont. *Science* **275**, 527–530.

Penmetsa, R. V., Frugoli, J. A., Smith, L. S., Long, S. R. and Cook, D. R. (2003). Dual genetic pathways controlling nodule number in *Medicago truncatula*. *Plant Physiology* **131**, 998–1008.

Peragine, A., Yoshikawa, M., Wu, G., Albrecht, H. L. and Poethig, R. S. (2004). SGS3 and SGS2/SDE1/RDR6 are required for juvenile development and the production of trans-acting siRNAs in Arabidopsis. *Genes & Development* **18**, 2368–2379.

Pereira, P. A. and Bliss, F. A. (1989). Selection of common bean (*Phaseolus vulgaris* L.) for Nitrogen fixation at differents levels of avaiable phosphorus under field and environmentally-controlled conditions. *Plant Soil* **115**, 75–82.

Raghothama, K. G. (1999). Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 665–693.

Rajagopalan, R., Vaucheret, H., Trejo, J. and Bartel, D. P. (2006). A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. *Genes & Development* **20**, 3407–3425.

Rashotte, A. M., Mason, M. G., Hutchison, C. E., Ferreira, F. J., Schaller, G. E. and Kieber, J. J. (2006). A subset of Arabidopsis AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 11081–11085.

Reed, R. C., Brady, S. R. and Muday, G. K. (1998). Inhibition of auxin movement from the shoot into the root inhibits lateral root development in Arabidopsis. *Plant Physiology* **118**, 1369–1378.

Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R. and Ruvkun, G. (2000). The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* **403**, 901–906.

Reinhart, B. J., Weinstein, E. G., Rhoades, M. W., Bartel, B. and Bartel, D. P. (2002). MicroRNAs in plants. *Genes & Development* **16**, 1616–1626.

Remans, T., Nacry, P., Pervent, M., Girin, T., Tillard, P., Lepetit, M. and Gojon, A. (2006). A central role for the nitrate transporter NRT2.1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. *Plant Physiology* **140**, 909–921.

Remington, D. L., Vision, T. J., Guilfoyle, T. J. and Reed, J. W. (2004). Contrasting modes of diversification in the Aux/IAA and ARF gene families. *Plant Physiology* **135**, 1738–1752.

Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B. and Bartel, D. P. (2002). Prediction of plant microRNA targets. *Cell* **110**, 513–520.

Riefler, M., Novak, O., Strnad, M. and Schmulling, T. (2006). Arabidopsis cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *Plant Cell* **18**, 40–54.

Rubio, V., Linhares, F., Solano, R., Martin, A. C., Iglesias, J., Leyva, A. and Paz-Ares, J. (2001). A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes & Development* **15**, 2122–2133.

Scheres, B., Di Laurenzio, L., Willemsen, V., Hauser, M. T., Janmaat, K., Weisbeek, P. and Benfey, P. (1995). Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis. *Development* **121**, 53–62.

Shin, R., Berg, R. H. and Schachtman, D. P. (2005). Reactive oxygen species and root hairs in Arabidopsis root response to nitrogen, phosphorus and potassium deficiency. *Plant Cell Physiology* **46**, 1350–1357.

Sieberer, T. and Leyser, O. (2006). Plant science. Auxin transport, but in which direction? *Science* **312**, 858–860.

Signora, L., De Smet, I., Foyer, C. H. and Zhang, H. (2001). ABA plays a central role in mediating the regulatory effects of nitrate on root branching in Arabidopsis. *The Plant Journal* **28**, 655–662.

Singh, M. B. and Bhalla, P. L. (2006). Plant stem cells carve their own niche. *Trends in Plant Science* **11**, 241–246.

Singleton, P. W. and Bohlool, B. (1984). Effect of salinity on nodule formation by soybean. *Plant Physiology* **74**, 72–76.

Sorin, C., Bussell, J. D., Camus, I., Ljung, K., Kowalczyk, M., Geiss, G., McKhann, H., Garcion, C., Vaucheret, H., Sandberg, G. and Bellini, C. (2005). Auxin and light control of adventitious rooting in Arabidopsis require ARGONAUTE1. *Plant Cell* **17**, 1343–1359.

Souter, M. A., Pullen, M. L., Topping, J. F., Zhang, X. and Lindsey, K. (2004). Rescue of defective auxin-mediated gene expression and root meristem function by inhibition of ethylene signalling in sterol biosynthesis mutants of Arabidopsis. *Planta* **219**, 773–783.

Stacey, G., Libault, M., Brechenmacher, L., Wan, J. and May, G. D. (2006). Genetics and functional genomics of legume nodulation. *Current Opinion in Plant Biology* **9**, 110–121.

Stepanova, A. N., Hoyt, J. M., Hamilton, A. A. and Alonso, J. M. (2005). A link between ethylene and auxin uncovered by the characterization of two root-specific ethylene-insensitive mutants in Arabidopsis. *Plant Cell* **17**, 2230–2242.

Sunkar, R. and Zhu, J. K. (2004). Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. *Plant Cell* **16**, 2001–2019.

Sunkar, R., Kapoor, A. and Zhu, J. K. (2006). Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by down-regulation of miR398 and important for oxidative stress tolerance. *Plant Cell* **18**, 2051–2065.

Suzuki, A., Akune, M., Kogiso, M., Imagama, Y., Osuki, K., Uchiumi, T., Higashi, S., Han, S. Y., Yoshida, S., Asami, T. and Abe, M. (2004). Control of nodule number by the phytohormone abscisic acid in the roots of two leguminous species. *Plant and Cell Physiology* **45**, 914–922.

Swarup, R., Kramer, E. M., Perry, P., Knox, K., Leyser, H. M., Haseloff, J., Beemster, G. T., Bhalerao, R. and Bennett, M. J. (2005). Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nature Cell Biology* **7**, 1057–1065.

Tansengco, M. I., Hayashi, M., Kawaguchi, M., Imaizumi-Anraku, H. and Murooka, Y. (2003). crinkle, a novel symbiotic mutant that affects the infection thread growth and alters the root hair, trichome, and seed development in *Lotus japonicus*. *Plant Physiology* **131**, 1054–1063.

Teale, W. D., Paponov, I. A. and Palme, K. (2006). Auxin in action: Signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology* **7**, 847–859.

Timmers, A. C., Auriac, M. C. and Truchet, G. (1999). Refined analysis of early symbiotic steps of the Rhizobium-Medicago interaction in relationship with microtubular cytoskeleton rearrangements. *Development* **126**, 3617–3628.

To, J. P., Haberer, G., Ferreira, F. J., Deruere, J., Mason, M. G., Schaller, G. E., Alonso, J. M., Ecker, J. R. and Kieber, J. J. (2004). Type-A Arabidopsis response regulators are partially redundant negative regulators of cytokinin signaling. *Plant Cell* **16**, 658–671.

Todd, C. D., Zeng, P., Huete, A. M., Hoyos, M. E. and Polacco, J. C. (2004). Transcripts of MYB-like genes respond to phosphorous and nitrogen deprivation in Arabidopsis. *Planta* **219**, 1003–1009.

Tuberosa, R., Salvi, S., Sanguineti, M. C., Landi, P., Maccaferri, M. and Conti, S. (2002a). Mapping QTLs regulating morpho-physiological traits and yield: Case studies, shortcomings and perspectives in drought-stressed maize. *Annals of Botany (London)* **89**(Spec No), 941–963.

Tuberosa, R., Sanguineti, M. C., Landi, P., Giuliani, M. M., Salvi, S. and Conti, S. (2002b). Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. *Plant Molecular Biology* **48**, 697–712.

Ulmasov, T., Hagen, G. and Guilfoyle, T. J. (1999). Activation and repression of transcription by auxin-response factors. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 5844–5849.

van der Weele, C. M., Spollen, W. G., Sharp, R. E. and Baskin, T. I. (2000). Growth of *Arabidopsis thaliana* seedlings under water deficit studied by control of water potential in nutrient-agar media. *Journal of Experimental Botany* **51**, 1555–1562.

Vanneste, S., De Rybel, B., Beemster, G. T., Ljung, K., De Smet, I., VanIsterdael, G., Naudts, M., Iida, R., Gruissem, W., Tasaka, M., Inze, D., Fukaki, H., *et al*. (2005). Cell cycle progression in the pericycle is not sufficient for SOLITARY ROOT/IAA14-mediated lateral root initiation in *Arabidopsis thaliana*. *Plant Cell* **17**, 3035–3050.

Vartanian, N. (1981). Some aspects of structural and functional modifications induced by drought in root systems. *Plant Soil* **63**, 83–92.

Vartanian, N., Marcotte, L. and Giraudat, J. (1994). Drought rhizogenesis in *Arabidopsis thaliana* (differential responses of hormonal mutants). *Plant Physiology* **104**, 761–767.

Vazquez, F., Gasciolli, V., Crete, P. and Vaucheret, H. (2004a). The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. *Current Biology* **14**, 346–351.

Vazquez, F., Vaucheret, H., Rajagopalan, R., Lepers, C., Gasciolli, V., Mallory, A. C., Hilbert, J. L., Bartel, D. P. and Crete, P. (2004b). Endogenous trans-acting siRNAs regulate the accumulation of Arabidopsis mRNAs. *Molecular Cell* **16**, 69–79.

Veereshlingam, H., Haynes, J. G., Penmetsa, R. V., Cook, D. R., Sherrier, D. J. and Dickstein, R. (2004). nip, a symbiotic *Medicago truncatula* mutant that forms root nodules with aberrant infection threads and plant defense-like response. *Plant Physiology* **136**, 3692–3702.

Vernoux, T., Wilson, R. C., Seeley, K. A., Reichheld, J. P., Muroy, S., Brown, S., Maughan, S. C., Cobbett, C. S., Van Montagu, M., Inze, D., May, M. J. and Sung, Z. R. (2000). The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* **12**, 97–110.

Voinnet, O. (2005). Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* **6**, 206–220.

Walch-Liu, P., Ivanov, II., Filleur, S., Gan, Y., Remans, T. and Forde, B. G. (2006). Nitrogen regulation of root branching. *Annals of Botany (London)* **97**, 875–881.

Wang, B. and Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* **16**, 299–363.

Wang, J. W., Wang, L. J., Mao, Y. B., Cai, W. J., Xue, H. W. and Chen, X. Y. (2005). Control of root cap formation by MicroRNA-targeted auxin response factors in Arabidopsis. *Plant Cell* **17**, 2204–2216.

Werner, T., Motyka, V., Strnad, M. and Schmulling, T. (2001). Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 10487–10492.

Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H. and Schmulling, T. (2003). Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *Plant Cell* **15**, 2532–2550.

Wightman, B., Ha, I. and Ruvkun, G. (1993). Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* **75**, 855–862.

Williams, L., Carles, C. C., Osmont, K. S. and Fletcher, J. C. (2005a). A database analysis method identifies an endogenous trans-acting short-interfering RNA that targets the Arabidopsis ARF2, ARF3, and ARF4 genes. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 9703–9708.

Wilmoth, J. C., Wang, S., Tiwari, S. B., Joshi, A. D., Hagen, G., Guilfoyle, T. J., Alonso, J. M., Ecker, J. R. and Reed, J. W. (2005). NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *The Plant Journal* **43**, 118–130.

Winicov, I. (1993). cDNA encoding putative zinc finger motifs from salt-tolerant alfalfa (*Medicago sativa* L.) cells. *Plant Physiology* **102**, 681–682.

Winicov, I. (2000). Alfin transcriptor factor overexpression enhances plants root growth under normal and saline conditions and improves salt tolerance in alfalfa. *Planta* **210**, 416–422.

Wopereis, J., Pajuelo, E., Dazzo, F. B., Jiang, Q., Gresshoff, P. M., De, B. F. J., Stougaard, J. and Szczyglowski, K. (2000). Short root mutant of *Lotus japonicus* with a dramatically altered symbiotic phenotype. *The Plant Journal* **23**, 97–114.

Wysocka-Diller, J. W., Helariutta, Y., Fukaki, H., Malamy, J. E. and Benfey, P. N. (2000). Molecular analysis of SCARECROW function reveals a radial patterning mechanism common to root and shoot. *Development* **127**, 595–603.

Xie, Q., Frugis, G., Colgan, D. and Chua, N. H. (2000). Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes & Development* **14**, 3024–3036.

Xiong, L., Wang, R. G., Mao, G. and Koczan, J. M. (2006). Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiology* **142**, 1065–1074.

Yang, L., Liu, Z., Lu, F., Dong, A. and Huang, H. (2006). SERRATE is a novel nuclear regulator in primary microRNA processing in Arabidopsis. *The Plant Journal* **47**, 841–850.

Zhang, B., Pan, X., Cannon, C. H., Cobb, G. P. and Anderson, T. A. (2006). Conservation and divergence of plant microRNA genes. *The Plant Journal* **46**, 243–259.

Zhang, H. and Forde, B. G. (1998). An Arabidopsis MADS box gene that controls nutrient-induced changes in root architecture. *Science* **279**, 407–409.

Zhang, H., Jennings, A., Barlow, P. W. and Forde, B. G. (1999). Dual pathways for regulation of root branching by nitrate. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 6529–6534.