Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource

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Abstract

Background Nitrogen (N) is one of the key mineral nutrients for plants and its availability has a major impact on their growth and development. Most often N resources are limiting and plants have evolved various strategies to modulate their root uptake capacity to compensate for both spatial and temporal changes in N availability in soil. The main N sources for terrestrial plants in soils of temperate regions are in decreasing order of abundance, nitrate, ammonium and amino acids. N uptake systems combine, for these different N forms, high- and low-affinity transporters belonging to multige families. Expression and activity of most uptake systems are regulated locally by the concentration of their substrate, and by a systemic feedback control exerted by whole-plant signals of N status, giving rise to a complex combinatory network. Besides modulation of the capacity of transport systems, plants are also able to modulate their growth and development to maintain N homeostasis. In particular, root system architecture is highly plastic and its changes can greatly impact N acquisition from soil.

Scope In this review, we aim at detailing recent advances in the identification of molecular mechanisms responsible for physiological and developmental responses of root N acquisition to changes in N availability. These mechanisms are now unravelled at an increasing rate, especially in the model plant *Arabidopsis thaliana* L.. Within the past decade, most root membrane transport proteins that determine N acquisition have been identified. More recently, molecular regulators in nitrate or ammonium sensing and signalling have been isolated, revealing common regulatory genes for transport system and root development, as well as a strong connection between N and hormone signalling pathways.

Conclusion Deciphering the complexity of the regulatory networks that control N uptake, metabolism and plant development will help understanding adaptation of plants to sub-optimal N availability and fluctuating environments. It will also provide solutions for addressing the major issues of pollution and economical costs related to N fertilizer use that threaten agricultural and ecological sustainability.

Keywords Nitrate · Ammonium · Urea · Nutrient sensing and signalling · Root uptake · Root development

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Introduction

A major challenge for sustainable agriculture will be to increase crop production with less, or at least no

more nitrogen (N) fertilizer input (Tilman et al. 2002; Good et al. 2004; Gross 2012). Increasing crop production is unavoidable first to keep pace with the demographic evolution that predicts approx.9 billions humans on Earth in 2050, and second to ensure part of the energy production through biofuels (Tilman et al. 2002; Rothstein 2007). Stabilizing, and if possible, decreasing N fertilizer utilization is also required to save fossil fuel and to prevent detrimental environmental consequences of excess N fertilizer application. Indeed, usage of N fertilizers is one of the most energy consuming processes associated with agriculture (Rothstein 2007, Xu et al. 2012), and its tremendous increase (9-fold world while since 1960, Hinsinger et al. 2011) is the main cause of a doubling in the annual input of reactive N species (all inorganic forms of N except N_2) in the environment, as compared to pre-industrial era (Canfield et al. 2010; Diaz and Rosenberg 2008; Galloway et al. 2003 and 2008). This major anthropogenic modification of the N cycle has now strongly harmful consequences, because only 30–50 % of the applied fertilizer N are actually captured by crops (Tilman et al. 2002), with most of the rest leaching to underground water primarily in the form of nitrate (NO_3^-), and/or being emitted in the atmosphere in the form of nitrogen oxides (N_xO_y) or ammonia (NH_3). These leaching processes are not exclusive of each other, and the same N atom may be in turn present in the atmosphere as a gas and in water as a solute as it moves from one environmental compartment to another during its cycling (the so-called "Nitrogen cascade", Galloway et al. 2003 and 2008). Pollution of ground, surface and coastal water by NO_3^- favours eutrophication and hypoxia, with dramatic consequences for the local ecosystems that can be heavily degraded (Diaz and Rosenberg 2008; Canfield et al. 2010; Galloway et al. 2003 and 2008). Emission of N gaseous forms increases: i) greenhouse effect in the atmosphere (in particular nitrous oxide, which has a greenhouse effect 300-fold higher than CO_2), ii) ozone concentration in the troposphere, and iii) formation of aerosols and fine particulate matter (Canfield et al. 2010; Galloway et al. 2003 and 2008).

As a consequence, improving N use efficiency by plants has emerged as a crucial goal for plant scientists, mostly through the generation of new crop varieties using genetic and/or biotechnological approaches (Good et al. 2004; Kant et al. 2011). There are several definitions of N use efficiency (Good et al. 2004; Xu

et al. 2012), and it is usual to discriminate between N acquisition efficiency (the proportion of N taken up as compared to that available in the soil), and N utilization efficiency (biomass production per unit N taken up by the plant). At first sight, these two components are associated with different physiological processes: more active uptake systems and improved soil exploration by the roots for N acquisition efficiency, and stimulated photosynthetic/metabolic activity as well as efficient N remobilisation between organs for N utilization efficiency. For both aspects, however, it is known for long that plants are able to achieve improved N use efficiency when facing N limiting conditions (Kant et al. 2011). Thus, it is important for future genetic/biotechnological strategies to identify the mechanisms plants have naturally evolved to adapt to low N availability.

This review aims at detailing the recent advances in the understanding of the physiological and molecular mechanisms that trigger adaptive responses of the plants to sub-optimal N availability, in particular those involved in maintaining optimal N nutrition through increased N acquisition efficiency. Given the complexity of this topic, we will not address the role of symbioses (with N_2 fixing bacteria or with mycorhizae) but concentrate only on processes occurring in plants ensuring their N nutrition through direct acquisition of N molecules by root cells from the soil solution. Therefore, a full section of this paper will be devoted to the description of the transport systems that allow these N molecules to be taken up by the roots. Plants react in many different ways to changes in N provision (for review: Clarkson 1985; Crawford and Glass 1998; Forde 2002; Hermans et al. 2006; Krouk et al. 2010a; Kant et al. 2011), but we will specifically focus on three types of responses for which key underlying mechanisms have been recently unravelled, namely: i) regulation of root N uptake systems, ii) plasticity of root system architecture, and iii) fast modulation of shoot growth. These three responses do not have the same goal. The first two generally result in an improved efficiency of root N uptake at low N availability, through both upregulation of specific high-affinity membrane transporters and enhanced foraging by the root system. The third one aims at quickly slowing down the overall N demand of the plant, to prevent N starvation when conditions for N uptake from the external medium are becoming unfavourable.

Despite these differences, all three responses share the common feature to be controlled by the combined

action of external and internal signals, that are often associated with local and systemic signalling pathways in the plant, respectively (Forde 2002; Gojon et al. 2009). Most important external signals are the extra-cellular concentrations of the N nutrient themselves, NO_3^- and NH_4^+ in particular, that are sensed locally by the roots (Crawford 1995; Stitt 1999; Tsay et al. 2011). Equally important signals are internal ones, especially those related to the overall N status of the whole organism that circulate between organs, and are transported downward to the roots to inform them of the N demand of the plant (Imsande and Touraine 1994; Liu et al. 2009). Taking into account the interplay between local and systemic signalling pathways is crucial for understanding many plant responses to N availability (Alvarez et al. 2012). For instance, on the one hand, systemic signalling of the whole plant N status is required to warrant that the roots will fulfill their specific function of N acquisition at the benefit of all organs. On the other hand, local signalling pathways are strictly mandatory for an autonomous behaviour of the various parts of the root system that allows overcoming the spatial heterogeneity of N concentration in the soil (Zhang et al. 1999; De Kroon et al. 2009). Furthermore, because local and systemic signalling pathways have often opposite actions on the same targets (e.g., local stimulation *vs* systemic repression of lateral root growth by NO_3^-, Zhang et al. 1999, see further details below in this review), their interplay explains why an apparently similar treatment (e.g., NO_3^- supply to the plants) may lead to confusing or contradictory responses. Indeed, the complex balance between the opposite effects of local and systemic signals may yield an outcome in one direction or the other, depending on the conditions. Therefore, for many responses to N availability addressed in this review, special attention will be paid to differentiate between regulatory mechanisms acting either at the local or systemic level, and to understand how their cross-talk results in a relevant integrated reaction of the plant.

Physiological and molecular bases of root N uptake

Plants can use a wide variety of chemical N forms, ranging from simple inorganic N compounds such as ammonium (NH_4^+) and nitrate (NO_3^-) to polymeric N forms such as proteins. However, it is generally assumed that in almost all ecosystems, plants take up mainly NH_4^+ and NO_3^-, rather than amino acids or other N organic forms, which apparently only play a role in extremely N-poor and cold ecosystems where N mineralization from soil organic matter is very limited (Marschner 1995; Schimel and Chapin 1996). However, this assumption now needs to be revisited, because urea has become the N fertilizer most widely used in agriculture on a global scale (http://faostat.fao.org), amounting to about half of total fertilizer N consumption. A widespread opinion is that most of the urea-N provided to crops is in fact taken up by plants in the form of NH_4^+, following fast microbial hydrolysis of urea in the soil, and that direct urea uptake and internal hydrolysis by the plant are not significant. However, direct urea incorporation in leaves can certainly contribute to N intake into the plants when fertilization is performed as foliar spray. Until now, research on plant N acquisition had a strong focus on uptake and assimilation of NO_3^- and NH_4^+, but physiological and molecular aspects of root uptake of urea and amino acids have recently received more attention.

The concentration of NH_4^+ in the soil is generally significantly lower than that of NO_3^- (Crawford and Glass 1998; von Wirén et al. 2000a). However, it is crucial to consider the acquisition of NH_4^+ alongside that of NO_3^-. Indeed, taking into account the use of urea as a main N fertilizer, it is likely that the importance of NH_4^+ as an N source has increased in agrosystems. Furthermore, even if pure NH_4^+ nutrition can lead to toxicity symptoms in many herbaceous plants, a balanced N diet (NO_3^- and NH_4^+) is clearly beneficial in many species as compared to pure NO_3^- nutrition (Forde and Clarkson 1999). Finally, even at low external concentration, NH_4^+ has strong effects on the expression and/or activity of root NO_3^- transport systems, thus playing a key role in the overall regulation of N acquisition by the plant.

Uptake of NO_3^- or NH_4^+ by the plant is the difference between influx into and efflux out of root cells (Crawford and Glass 1998; von Wirén et al. 2000a; Tsay et al. 2007). However, with the exception of specific stress conditions (e.g., Segonzac et al. 2007), net uptake of both ions is mostly determined by factors affecting influx (Lee 1993). Because most N transport proteins identified to date are either influx or efflux (and not bi-directional) transporters (Miller et al. 2007; Tsay et al. 2007; Wang et al. 2012), this suggests that

influx carriers are the main control points for N intake into the roots. The kinetics of root NO_3^- or NH_4^+ uptake (or influx) as a function of the external concentration generally display a bi-phasic pattern (Marschner 1995; Crawford and Glass 1998). In the low concentration range, high-affinity transport systems (HATS) are able to scavenge ions from the soil at concentrations as low as 1 μM. Both NO_3^- and NH_4^+ HATS are saturable, and their activity can be modelled using the Michaelis-Menten formalism (Wang et al. 1993; Filleur et al. 2001). In the high concentration range (typically >0.5–1 mM), the activity of low-affinity transport systems (LATS) becomes evident, superimposed to that of the HATS. Unlike with the HATS, the NO_3^- or NH_4^+ uptake (or influx) mediated by the LATS is not saturable, but generally shows a linear increase with increasing external concentration (Wang et al. 1993; Touraine and Glass 1997). This has often been considered as evidence that HATS and LATS do not involve the same transporter proteins. This general HATS/LATS duality for root N uptake has been consistently observed in almost all species investigated to date, and under most environmental conditions (Doddema et Telkamp 1979, Wang et al. 1993; Vidmar et al. 2000, Santi et al. 2003 Tong et al. 2005).

The relative contributions of HATS and LATS to the overall N uptake are clearly different between NO_3^- and NH_4^+. In most soils, free NH_4^+ is found at low concentrations, well below 1 mM (vonWirén et al. 2000a). Therefore, it is anticipated that for this N source, the HATS plays a predominant role over the LATS for nutrition of the plant. This is markedly different for NO_3^-, because its concentration in the soil solution fluctuates dramatically. From few micromolar, it can reach the range of 1–10 mM following fertilizer application or a burst in nitrification (Crawford and Glass 1998; Miller et al. 2007). Together with the differential regulation of HATS and LATS, this makes it difficult to determine the respective roles of each system in the cumulated root NO_3^- uptake by crops over long periods of time (Miller et al. 2007). Only very few attempts were made to compute HATS- or LATS-mediated NO_3^- uptake by crops over the growth cycle. Malagoli et al. (2004) found that, taking into account the seasonal pattern of NO_3^- concentration in the soil, the kinetics parameters of both HATS and LATS, as well as their regulation, the NO_3^- HATS had a major contribution to N acquisition by rapeseed grown in the field under regular agricultural practice.

Major insight into the individual NO_3^- and NH_4^+ carrier proteins responsible for N uptake by plant roots has been done recently (Tsay et al. 2007; Gojon et al. 2009). The main structural components of both NO_3^- and NH_4^+ HATS have been identified and functionally characterized in *Arabidopsis thaliana* (Fig. 1), and this work is now being carried out in several major crops. Additionally, low affinity NO_3^- transporters have been isolated and characterized. At the opposite, nothing is known at the molecular level concerning the LATS for NH_4^+.

Nitrate transporters

In higher plants, membrane NO_3^- transporters or channels identified to date belong to five protein families, namely NRT1, NRT2, CLC, ALMT and SLAC1 (Forde 2000; Tsay et al. 2007; Geiger et al. 2009; Gojon et al. 2009; Sasaki et al. 2010; Barbier-Brygoo et al. 2011). However, only members of the NRT1 and NRT2 families have been shown to be involved in root NO_3^- uptake (Fig. 1). Most of the NRT1/NRT2 transporters characterized are either high- or low-affinity transporters, thus supporting the general idea that HATS and LATS involve different carrier proteins. However, there are two exceptions to this functional separation, i.e., NRT1.1 of *A.thaliana* and NRT1.3 of *Medicago truncatula* L., which were both reported to display a dual affinity for NO_3^- (Wang et al. 1998; Liu et al. 1999; Morere-Le Paven et al. 2011).

High-affinity NO_3^- transporters were predominantly found in the NRT2 family, which comprises seven genes in *A.thaliana* (Orsel et al. 2002, Okamoto et al. 2003). Among them, NRT2.1 has been shown to be the major contributor, accounting on its own for up to 75 %, of the total HATS activity (Filleur et al. 2001; Li et al. 2007). Besides NRT2.1, two other members of the gene family, NRT2.2 and NRT2.4, were found to play a more minor role in the NO_3^- HATS (Li et al. 2007; Kiba et al. 2012). However, even if *NRT2.2* represents less than 20 % of the HATS activity, when *NTR2.1* is mutated, *NRT2.2* mRNA levels are increased up to three-fold to compensate the functional loss of *NRT2.1* (Li et al. 2007). Furthermore, NRT2.4 was characterized as a very high-affinity transporter, contributing significantly to the whole HATS activity at NO_3^- concentrations below 25 μM (Kiba et al. 2012), whereas NRT2.1 and NRT2.2 are major players at concentrations higher than 50 μM (Cerezo et al. 2001). Recent studies have suggested that NRT2.1 in *Chlamydomonas reinhardtii* (Zhou et al.

Fig. 1 Schematic representation of the membrane transporters involved in the root uptake of the four major sources of nitrogen in *Arabidopsis thaliana* plants. Proteins are grouped according to their substrate and to their affinity for the substrate (*HATS* High Affinity transporters and *LATS* Low affinity transporters). Transporters highlighted with big letters are those that play a major role in nutrient uptake. *Question marks* represent unconfirmed results. Several amino acid and peptide transporters have been identified in heterologous system but there *in planta* function have not yet been characterised. NRT1-1 is both a LATS and HATS depending on phosphorylation of the threonine 101 residue. NAR2.1 is a protein of unknown function that is mandatory to ensure nitrate transport by NRT2 transporters
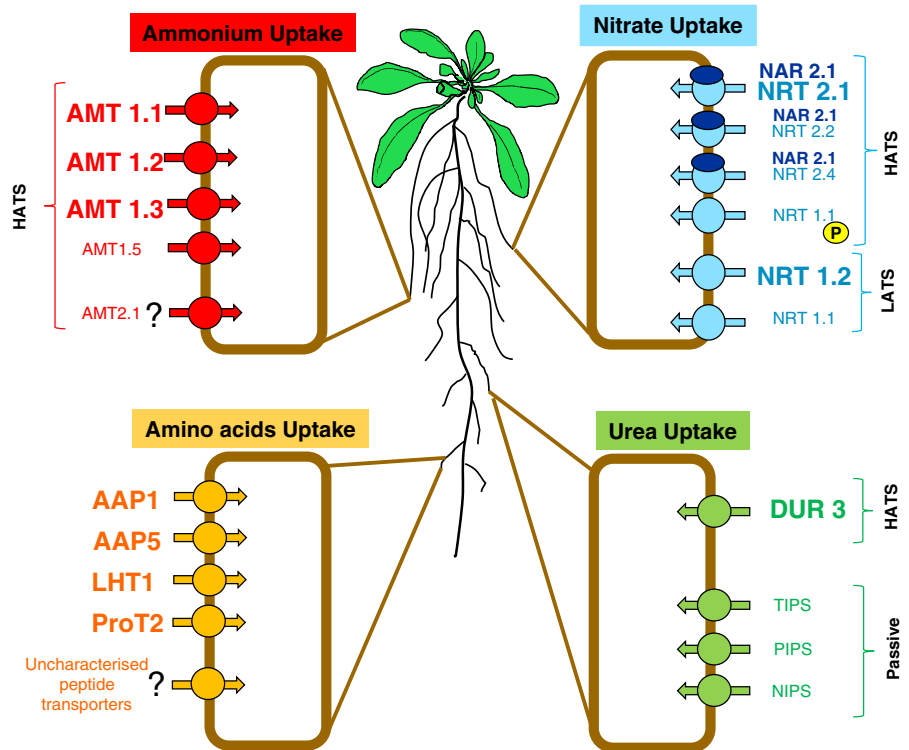
2000), barley (*Hordeum vulgare* L.) (Tong et al. 2005), and *A.thaliana* (Okamoto et al. 2006) require a second protein (NAR2/NRT3) to function as a NO_3^- transporter. NAR2 proteins have no known transport or catalytic activity, but in *A.thaliana*, NAR2.1 (NRT3.1) is strictly required for both expression and activity of NRT2.1 (Orsel et al. 2006; Wirth et al. 2007). Recently, Yong et al. (2010) observed that NRT2.1 and NAR2.1 proteins physically interact at the plasma membrane suggesting that the active transport system may in fact be a heterooligomer of NRT2.1 and NAR2.1. In *A.thaliana*, there are two *NAR2* genes but to date only NAR2.1 has been found involved in HATS. Interestingly, in a *nar2.1* null mutant, the HATS activity was more strongly reduced than in the *nrt2.1nrt2.2* double mutant (Okamoto et al. 2006) suggesting that NAR2.1 was probably required for functionality of other NRT2 transporters. Recently Kotur et al. (2012) found that co-expression into *Xenopus* oocytes of AtNRT2 genes together with AtNAR2.1 resulted in a significant increases of NO_3^- uptake over and above that resulting from single injections for the seven AtNRT2 genes.

NRT2 and NAR2 genes have now been identified in many other species, such as barley (Trueman et al. 1996; Vidmar et al. 2000), rice (Oryza sativa L.) (Plett et al. 2010; Feng et al. 2011), maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and *Brachypodium distachyon* L. (Plett et al. 2010), Soybean (*Glycine max* L.) (Amarasighe et al. 1998) and *M. truncatula* (Ruffel et al. 2008). In all cases where the genome sequence is available, a multigene family was found for NRT2 proteins (with at least 3–4 members). However, *NRT2* genes from grass and cereals seem to be relatively phylogenetically distant from those in *A. thaliana*, making it difficult to speculate about possible orthologues of the NRT2 transporters functionally charaterized in this model species (Plett et al. 2010).

Low affinity NO_3^- transporters belong to the large NRT/PTR (Peptide Transporter) family that also comprise nitrite, peptide or carboxylic acid transporters (Forde 2000; Tsay et al. 2007). In *A. thaliana*, 9 NRT/PTR proteins have been functionally characterized to date as NO_3^- transporters, out of the 53 members predicted from the genome sequence (Tsay et al. 2007; Gojon et al. 2009; Li et al. 2010; Wang and Tsay

2011). However, only NRT1.1 and NRT1.2 were shown to participate in root NO_3^- uptake.

The *A. thaliana* NRT1.1 (initially named *CHL1* that stands for *CHLorate resistant mutant1*) was not only the first NO_3^- transporter gene to be cloned in plants (Tsay et al. 1993), but is also the most extensively studied. Both *in planta* NO_3^- uptake assays in *nrt1.1* (*chl1*) mutants and in NRT1.1-injected *Xenopus* oocytes initially identified NRT1.1 as a low affinity NO_3^- transporter participating in the LATS (Doddema and Telkamp 1979; Tsay et al. 1993; Huang et al. 1996; Touraine and Glass 1997). However, Wang et al. (1998) later showed that high-affinity NO_3^- uptake by the HATS was also defective in *chl1* mutant. Furthermore, *Xenopus* oocytes expressing NRT1.1 actually exhibit two phases of NO_3^- uptake kinetics, indicating that this protein has a unique feature of a dual-affinity NO_3^- transporter (Liu et al. 1999). It displays high-affinity transport activity when phosphorylated at the T101 residue, while the non-phosphorylated form is a low-affinity transporter (Liu and Tsay 2003; Ho et al. 2009). On the contrary, NRT1.2 is a strict low-affinity NO_3^- transporter (Huang et al. 1999). It is located in the root epidermis and cortex, and underexpressors or knockout mutants have a reduced LATS activity (Huang et al. 1999; Krouk et al. 2006). Other contributors to the low-affinity root NO_3^- uptake in *A. thaliana* still await identification because NRT1.1 and NRT1.2 alone cannot account for the total LATS activity. Indeed, knockout mutation of *NRT1.2* results only in a limited inhibition of the LATS activity (Huang et al. 1999; Krouk et al. 2006), and *chl1* mutants display defects in either HATS or LATS activity only in a limited range of experimental conditions, such as mixed NH_4^+/NO_3^- nutrition where NH_4^+, and not NO_3^-, is actually the main N form taken up by the plant (Touraine and Glass 1997; Wang et al. 1998).

Close homologues of the *A.thaliana* NRT1 have been identified in cereals including rice (Lin et al. 2000; Tsay et al. 2007; Plett et al. 2010), maize (Santi et al. 2003; Plett et al. 2010), and barley (West et al. 1998; Vidmar et al. 2000; Tong et al. 2005), and in other species like Tomato (*Solanum lycopersicum* L.) (Lauter et al. 1996) or *M. truncatula* (Ruffel et al. 2008, Morère-Le Paven et al. 2011). However, most of the studies on the activity and regulation of NRT1 transport proteins have been conducted in *A. thaliana*, and a huge work of functional characterization remains to be done in other species.

Ammonium transporters

The root uptake of NH_4^+ involves the AMT family of high-affinity transporters (Fig. 1), which is part of a larger group of NH_4^+ permeases of the Ammonium Transporter/Methylammonium Permease/Rhesus (AMT/MEP/Rh) family (von Wirén and Merrick 2004). Heterologous expression of selected plant AMTs in yeast or *Xenopus* oocytes indicate they function as high-affinity transporters, most probably as NH_4^+ uniporters that transport NH_4^+ along the electrochemical potential gradient (Ninnemann et al. 1994; Gazzarini et al. 1999; Ludewig et al. 2002, 2003). *A. thaliana* possesses a family of six AMT-type NH_4^+ transporters, where AMT1.1–AMT1.5 belong to the AMT1 clade, while AMT2.1 is more closely related to the MEP/AMTB subfamily found in yeast and bacteria (Ludewig et al. 2001). With the exception of *AMT1.4*, all the *AMT* genes are expressed in roots (Gazzarini et al. 1999; Yuan et al. 2007, 2009). AMT1.1 and AMT1.3 are localized in the plasma membrane of epidermal and cortical root cells and have each been shown to be individually responsible for ~30 % of the NH_4^+ HATS uptake capacity (Kaiser et al. 2002; Loqué et al. 2006). AMT1.2 is a lower affinity transporter expressed in endodermal and cortical cells and is thought to play a role either in uptake and retrieval of ammonium from the root apoplast (Yuan et al. 2007) or in NH_4^+ transfer into the vasculature (Neuhäuser et al. 2007). AMT2.1 is also expressed in the vasculature, cortical and root tip cells, but seems to play a marginal role in NH_4^+ uptake from the soil solution (Sohlenkamp et al. 2000; Yuan et al. 2007). Finally, Yuan et al. (2007), using a quadruple mutant knockout (*qko*) for *AMT1.1, AMT1.2, AMT1.3* and *AMT2.1,* and transgenic lines complemented with the individual *AMT* genes, showed that most of the NH_4^+ HATS capacity of N-starved wild type plants is accounted for by a relatively similar and additive contribution of AMT1.1, AMT1.2 and AMT1.3, with only 5–10 % of this capacity possibly attributable to AMT1.5 that is also localized in the plasma membrane of the epidermal cells, including root hairs (Yuan et al. 2007).

The AMT family has also been investigated in other species than *A. thaliana*, such as tomato (Lauter et al. 1996), rice (Kumar et al. 2003), *Lotus japonicus* L. (D'Apuzzo et al. 2004), and *Citrus sinensis* L. (Camanes et al. 2007). In rice, 10 putative *OsAMT* transporter genes were found, distributed into four different sub-families, *OsAMT1* to *OsAMT4* (Suenaga et

al. 2003). To date, the three *OsAMT1* genes have been most studied, and found to be significantly or predominantly expressed in the roots (Kumar et al. 2003; Sonoda et al. 2003a). Overexpression of *OsAMT1.1* sometimes resulted in an enhanced NH_4^+ influx in the roots, depending on genotype background and experimental conditions, thus supporting its role in NH_4^+ acquisition by the plant (Hoque et al. 2006; Kumar et al. 2006). However, *OsAMT1.1* overexpressors often show reduced growth as compared to wildtype plants when supplied with NH_4^+ as sole N source, possibly indicating toxicity effects of excessive NH_4^+ intake (Hoque et al. 2006; Kumar et al. 2006).

Organic N transporters

The quantitative contribution of organic N sources in the soil to plant N nutrition is still under debate (Nasholm et al. 2009) but all tested plant species have been found to possess the capacity to take up urea and amino acids. *A. thaliana* plants possess a high-affinity urea transporter (DUR3, Fig. 1) that is involved in taking up environmental urea but may also mediate internal urea transport (Liu et al. 2003a; Kojima et al. 2007). DUR3 was identified by its similarity to the urea transporter of *Saccharomyces cerevisiae* (ScDUR3), and was shown to account for most of the root urea HATS activity (Kojima et al. 2007). Orthologs of AtDUR3 are present throughout the green lineage from algae to higher plants, mostly encoded by single copy genes (Wang et al. 2008). The protein is expressed at the plasma membrane of root epidermal cells especially in nitrogen-starved plants and the promoter is responsive to urea in the absence of other nitrogen sources which is consistent with its role as urea transporter (Kojima et al. 2007; Merigout et al. 2008). Passive urea transport mediated by aquaporins (TIPs PIPs and NIPs) has been reported (Witte 2011). According to their location in the tonoplast membrane, TIPs proteins might be involved in urea storage in vacuoles rather than in uptake from soil (Liu et al. 2003b). On the contrary, two NIPS (At2g29870 and At2g34390), that are among the few genes that are specifically upregulated by urea, have been proposed to participate to soil urea uptake (Merigout et al. 2008) but further characterization remains to be done to better understand their function.

Early work initiated in the 1980s suggested that amino acid transport in plants was mediated either by a single system (Reihnold and Kaplan 1984), or by two major transport systems for neutral/acidic or basic amino acids, respectively (Datko and Mudd 1985; Schobert and Komor 1987). Later on, molecular approaches initiated by Frommer et al. (1993) who cloned a plant amino acid transporter gene (AAP1), and availability of full genome sequences, have allowed to grasp the abundance of amino acid transporters in plant genomes. They belong to at least five gene families, comprising no less than 67, 134 and 96 putative amino acid transporter genes in *A. thaliana*, Poplar (*Populus trichocarpa* L.) and rice, respectively (rewieved by Rentsch et al. 2007). The *in planta* function of this multitude of different transporters is far from being characterized (Tegeder 2012). However, AAP1 (Lee et al. 2007), AAP5 (Svennerstam et al. 2008), LHT1 (Svennerstam et al. 2007) and ProT2 (Lehmann et al. 2011) have been identified as crucial components of amino acid root uptake in *A. thaliana*, within the concentration ranges relevant for field conditions. Efflux of amino acids from roots has been shown to occur in several species (Paynel et al. 2001; Phillips et al. 2004, 2006; Lesuffleur et al. 2007). The molecular mechanisms involved remain to be elucidated. Hypothetically, energy-dependent carriers in the plasma membrane may mediate this transport. However, amino acid efflux may simply represent leakage from roots, as a result of the high concentration gradient across the plasmalemma (e.g., 1–10 mM inside the cell against 0.1–10 μM outside, Näsholm et al. 2009). In this context, it has been suggested that root amino acid uptake systems may primarily be involved in retrieval of amino acids that have leaked out of the cells (Jones et al. 2005).

Regulatory mechanisms affecting root N uptake systems

Root N uptake is strongly regulated in response to changes in the external N availability or in the N demand of the whole plant. Many of the transport systems detailed above are subjected to such regulation. However, a general conclusion arising from most studies is that the HATS for the various N sources are particularly responsive, and display a much higher flexibility than the corresponding LATS. There are two major mechanisms that co-ordinately act to modulate root N uptake as a function of external N supply or internal N demand (Crawford and Glass 1998;

Gojon et al. 2009). The first one relates to the direct effect of the N sources themselves that are perceived by specific sensing systems (e.g., NO_3^- or NH_4^+ acting as signal molecules). Depending on the transport system, this may lead to the induction or the repression of the uptake systems. The second mechanism is a feedback repression exerted by high N status of the whole plant, that downregulates high-affinity uptake systems under N satiety conditions. This repression is relieved when plants experience N limitation, resulting in a strong increase in HATS capacity that improves N uptake efficiency.

Induction of transporter gene expression by NO_3^-, NH_4^+ or urea

Nitrate is not only a nutrient but also acts as a potent signal molecule governing many aspects of plant physiology and development (Crawford 1995; Stitt 1999; Krouk et al. 2010a). A striking illustration of the signalling action of NO_3^- is that its supply to plants grown on other N sources, or previously N-starved for several days, results in a massive reprogrammation of the genome expression (Wang et al. 2000, 2003; Scheible et al. 2004). Up to 10 % of the transcriptome is responsive to NO_3^- (Krouk et al. 2010a), and for many genes, it has been clearly established that NO_3^- per se is the signal, because these genes are still NO_3^--regulated in mutants deficient in nitrate reductase, the first enzyme of the NO_3^- assimilation pathway (Wang et al. 2004). An exhaustive analysis of the far-reaching consequences of NO_3^- signalling on plant physiology is beyond the scope of this review. However, many studies have highlighted that major targets of NO_3^- signalling pathways are the transport systems participating in root NO_3^- uptake (Forde 2002; Tsay et al. 2007; Gojon et al. 2009). It is known for 40 years that NO_3^- supply leads to an induction of root NO_3^- uptake in various plant species. This was first documented by Jackson et al. (1972) who observed in wheat (*Triticum aestivum* L.), maize and tobacco (*Nicotiana tabacum* L.) that NO_3^- uptake rate was gradually increasing upon NO_3^- supply. Extensive molecular studies in *A. thaliana* have shown that this is associated with the induction of several NO_3^- transporter genes (Fig. 2), especially those encoding components of the HATS such as *NRT1.1*, *NRT2.1* and *NRT2.2* (Tsay et al. 1993; Filleur and Daniel-vedele 1999; Lejay et al. 1999; Zhuo et al. 1999; Okamoto et al. 2003). A similar feature has also been reported in

other plant species such as tomato (Lauter et al. 1996), soybean (Amarasinghe et al. 1998), barley (Vidmar et al. 2000), maize (Santi et al. 2003) or rice (Feng et al. 2011). However, uninduced plants still keep a basal capacity to take up NO_3^- in the low concentration range, and not all *NRT2* genes are induced by NO_3^- (Crawford and Glass 1998; Okamoto et al. 2003; Kiba et al. 2012). This gave rise to the hypothesis that the NO_3^- HATS comprises in fact two different components, a constitutive HATS (cHATS), present and active even in the absence of NO_3^-, and an inducible (iHATS) that is only expressed or activated after NO_3^- pretreatment. In *A. thaliana*, NRT2.1 plays a predominant role in the iHATS because null *nrt2.1* mutants have lost most of the stimulation of high-affinity NO_3^- uptake by NO_3^- (Cerezo et al. 2001; Li et al. 2007). Induction of NO_3^- transporter genes by NO_3^- is fast, being most often recorded as soon as 20–30 min after NO_3^- supply (Tsay et al. 1993; Wang et al. 2000; Scheible et al. 2004). It is well established from "split-root" experiments (in which only a part of the root system can be supplied with NO_3^-) that induction by NO_3^- is a local process (Burns 1991; Robinson 1994; Ohlen and Larsson 1992; Gansel et al. 2001). Although it can be triggered by NO_3^- concentrations as low as 10 μM, it is concentration-dependent, in a sometimes complex manner. For *NRT2.1*, two phases can be distinguished that closely mimic those found for NO_3^- uptake in the roots (Hu et al. 2009; Ho et al. 2009): a 'high-affinity' phase that leads to a moderate induction of the gene in the low NO_3^- concentration range, and a 'low-affinity phase', resulting in a further increase in *NRT2.1* expression at high NO_3^- concentration. In contrast to the HATS, the NO_3^- LATS shows little induction in response to NO_3^- supply (Crawford and Glass 1998). Accordingly, only the dual-affinity *NRT1.1* transporter gene has been reported to be NO_3^- inducible in *A. thaliana*, while other *NRT1* genes, including *NRT1.2* that encodes an important component of the LATS, were found to be constitutively expressed, regardless of the presence or absence of NO_3^- (Huang et al. 1999; Okamoto et al. 2003).

The molecular mechanisms involved in the NO_3^- regulation of NO_3^- transporter genes are far from being understood but at least three genes, namely *NRT1.1*, *NLP7* and *CIPK8*, were shown to be required for proper induction of NO_3^- transporter genes in *A. thaliana* (Fig. 3).

Quite unexpectedly, the NRT1.1 NO_3^- transporter plays a pivotal role in many NO_3^- signalling responses

(Fig. 3), supporting the hypothesis that it may in fact be a NO_3^- 'transceptor', i.e., a protein ensuring a dual transport and sensing function (Gojon et al. 2011). In particular, NRT1.1 was found to trigger the induction of *NRT2.1* expression by NO_3^- (Ho et al. 2009; Wang et al. 2009). Knockout mutation of *NRT1.1* almost totally prevents the increase in NRT2.1 mRNA accumulation in response to provision of NO_3^- for 30 min, following growth of the plants on NH_4^+-succinate as sole N source (Ho et al. 2009). However, this does not lead to a complete lack of *NRT2.1* expression in *nrt1.1* mutants. First, *NRT1.1* mutation only delays induction of *NRT2.1* that is effective after several hours as compared to 0.5–1 h in wild type plants. Second, the involvement of NRT1.1 in *NRT2.1* induction appears to be dependent on prior experimental conditions. Indeed, *nrt1.1* mutants display an unaffected induction of *NRT2.1* expression as compared to wild type plants when the NO_3^- treatment is performed on plants previously subjected to N deprivation for several days indicating that NRT1.1 is not the only NO_3^- sensor that regulates *NRT2-1* expression (Muños et al. 2004; Wang et al. 2009). A convincing evidence supporting the direct role of NRT1.1 as a NO_3^- sensor is the fact that point mutations in the protein uncouple transport and signalling activities. For instance, the P492L mutation suppresses NO_3^- transport by NRT1.1, but not the NRT1.1-dependent induction of *NRT2.1* by NO_3^- (Ho et al. 2009). Also, the T101A mutation that prevents phosphorylation of the protein and inhibits NO_3^- transport by NRT1.1 at low NO_3^- concentrations (Liu and Tsay 2003) actually stimulates *NRT2.1* induction under the same range of NO_3^- concentrations (Ho et al. 2009). These data show that there is no direct relationship between NO_3^- signalling and NO_3^- transport by NRT1.1 and that loss of NO_3^- signalling in *nrt1.1* KO mutants is not due to impaired uptake of the NO_3^- signal molecule. The actual mechanism by which NRT1.1 triggers NO_3^- signalling for induction of the *NRT2.1* gene remains however to be elucidated.

NLP7 is a putative transcription factor of the NIN-like (Nodule-INception-like) family of *A. thaliana*. These proteins are homologous to the NIT2 transcription factor of *C. reinhardtii* that was shown to be a main player in NO_3^- signalling, strictly required for induction of the nitrate reductase *NIA* gene (Camargo et al. 2007). Among the 7 members of the NLP family, NLP7 appears to be specifically involved in NO_3^- signalling, and necessary for full induction of *NIA1*,

NIA2, *NRT2.1* and *NRT2.2* genes (Castaings et al. 2009). However, the NO_3^- response of these genes is not totally impaired in *nlp7* knockout mutants, suggesting that unlike in *C. reinahdtii*, NO_3^- induction of gene expression in plants is not dependent on a single signalling pathway.

Finally, CIPK8 is a calcineurin B-like interacting protein kinase, which is inducible by NO_3^- at the gene level in a partially NRT1.1-dependent manner (Hu et al. 2009). Interestingly, CIPK8 also contributes to the induction by NO_3^- of several NO_3^- uptake and assimilation-related genes, including *NRT2.1* and *NRT1.1*. This mutual dependence of NRT1.1 and CIPK8 for their NO_3^- induction suggests a positive feedback loop in the NO_3^- signalling pathway governing expression of NO_3^--responsive genes.

It is still unclear whether NH_4^+ *per se* also has a similar signalling action in inducing its own transport systems (AMTs). Like that of NO_3^-, root uptake of NH_4^+ has been reported to be transiently stimulated after reintroduction of NH_4^+ following a period of low N provision (Mack and Tischner 1994). However, in most plant species investigated to date, expression of *AMT* genes is repressed rather than induced by NH_4^+ (see below), but there are exceptions to this general trend. In tomato for instance, while *LeAMT1.1* mRNA is most abundant when N availability is limited, transcription of *LeAMT1.2* is clearly upregulated by the supply of NH_4^+ (Lauter et al. 1996, Von Wiren et al. 2000b). In rice, expression of both *OsAMT1;1* and *OsAMT1;2* is stimulated by NH_4^+, and accordingly repressed by N starvation (Sonoda et al. 2003a). Nevertheless, this does not appear to be due to specific NH_4^+ signalling, because glutamine had the same positive effect and glutamine synthetase inhibitors prevented *OsAMT1;1* and *OsAMT1;2* induction by NH_4^+. Thus, these two genes seem to be regulated by downstream metabolites of NH_4^+ assimilation, possibly by glutamine itself since glutamine concentration in root tissues correlated well with *OsAMT1s* expression (Sonoda et al. 2003b). These authors (Sonoda et al. 2003b) proposed that the difference might come from the fact that contrary to *A. thaliana* and tomato, rice is adapted to flooded and hypoxic medium, so that NH_4^+ nutrition prevails. Concerning urea, molecular analysis of *AtDUR3* expression showed that its expression is downregulated by NO_3^- and NH_4^+, but markedly induced by supply of urea (Fig. 3, Kojima et al. 2007). Yet no further progress had been done to delineate this complex regulation.

Repression of transporter gene expression by N metabolites

A major regulation of root N uptake systems is their feedback repression by signals of N satiety. Indeed, numerous studies on various plant species have shown that the root uptake capacity for NO_3^-, NH_4^+ and urea is strongly downregulated when plants are subjected to ample N provision, whereas N starvation or N limitation results in the opposite response (Crawford and Glass 1998, von Wirén et al. 2000a, b; Kojima et al. 2007; Tsay et al. 2007; Gojon et al. 2009). Very little is known about any regulation of organic N uptake. However, studies based on mixed (organic/inorganic) N nutrition showed that uptake rates of amino acids are very little affected by N treatments, compared to NO_3^- and NH_4^+ uptake rates, that were strongly responsive both in soil (Ohlund and Nasholm 2001) or in solution (Thornton and Robison 2005). Concerning root NO_3^- transport systems, split-root experiments have demonstrated that this regulation relies on systemic signalling pathways, modulating NO_3^- uptake rate as a function of the N status of the whole plant. For instance, N deprivation of one side of the split-root system leads to a compensatory stimulation of NO_3^- uptake by the other side of the root system that remains under unmodified NO_3^- supply (Burns 1991; Robinson 1994; Ohlen and Larsson 1992; Gansel et al. 2001). As long as the proportion of the root system subjected to N deprivation does not remain too high (e.g., <50–75 %), the other roots can within a few days fully compensate for the lack of NO_3^- uptake by N-starved roots, to restore total N acquisition at the level measured on the whole root system prior to the localized N deprivation treatment (Gansel et al. 2001; Ruffel et al. 2008). Therefore, even in roots well fed with N, NO_3^- uptake systems can react to the N starvation experienced by other organs, indicating long-distance signalling of N limitation. This regulation is thought to have a crucial physiological significance because it is certainly the means by which plants are able to rapidly adjust their N uptake efficiency according to the changes in their N demand for growth.

For both NO_3^- and NH_4^+, the HATS appears to be the main target of the feedback regulation by the N status of the plant. Indeed, unlike that of the HATS, the LATS activity is generally not markedly repressed or stimulated by high or low N supply, respectively (Wang et al. 1993; Lejay et al. 1999). Molecular data are in agreement with this conclusion. In most species

investigated, specific members of the NRT2 and AMT1 families of high-affinity transporters are strongly modulated at the transcript level in response to the changes in N status of the plant (Fig. 2). In *A. thaliana*, *NRT2.1*, *NRT2.2*, *AMT1.1*, *AMT1.2* and *AMT1.3* genes are all strongly derepressed by N starvation, with an increase in mRNA level in the roots that can be >10-fold as compared to that in well-fed plants (Gazzarini et al. 1999; Lejay et al. 1999; Zhuo et al. 1999). This is also true for the high-affinity urea transporter gene *DUR3* (Kojima et al. 2007). Moreover, most of these genes were shown to be actually responsible for the modulation of the HATS by the N demand of the plant. Indeed, knockout mutants for both *NRT2.1* and *NRT2.2*, or for the three *AMT1.1-3* genes show little increase in NO_3^- or NH_4^+ HATS activity, respectively, in response to N starvation (Cerezo et al. 2001; Loqué et al. 2006; Yuan et al. 2007). For *NRT2.1*, *AMT1.1*, *AMT1.3* and *DUR3*, promoter-reporter gene fusions showed that regulation occurs at the transcriptional level (Nazoa et al. 2003; Loqué et al. 2006; Kojima et al. 2007; Girin et al. 2007, 2010). For NRT2.1, split-root experiments indicated that regulation of its expression is due to systemic signalling, since this gene is upregulated in roots still fed with NO_3^- in response to a localized N starvation treatment on another portion of the root system (Gansel et al. 2001). Studies on other species than *A. thaliana* have confirmed that *NRT2* and *AMT1* genes are under feedback repression by high N status of the plant: *HvNRT2.x* in barley (Vidmar et al. 2000), *NpNRT2.1* in *Nicotiana Plumbaginifolia* L. (Krapp et al. 1998), *ZmRNT2.1* in maize (Santi et al. 2003), *OsNRT2.* genes in rice (Cai et al. 2008), *LeNRT2.* genes in tomato (Lauter et al. 1996) or *MtNRT2.* genes in *M. truncatula* (Ruffel et al. 2008). Concerning the low-affinity NO_3^- transporters genes, neither *NRT1.1* nor *NRT1.2* was shown to be upregulated by N starvation in *A thaliana* (Huang et al. 1999; Lejay et al. 1999), in agreement with the observations based on measurements of the NO_3^- LATS activity..

Although the systemic control exerted on root NO_3^- and NH_4^+ transporters has been clearly defined at the physiological level, and further documented by the identification of the transporter genes targeted by this control, almost nothing is known at the molecular level concerning the regulatory mechanisms themselves. To date, only a single gene, namely *HNI9/IWS1* of *A. thaliana*, was shown to participate in the signalling

Induction   **Repression**
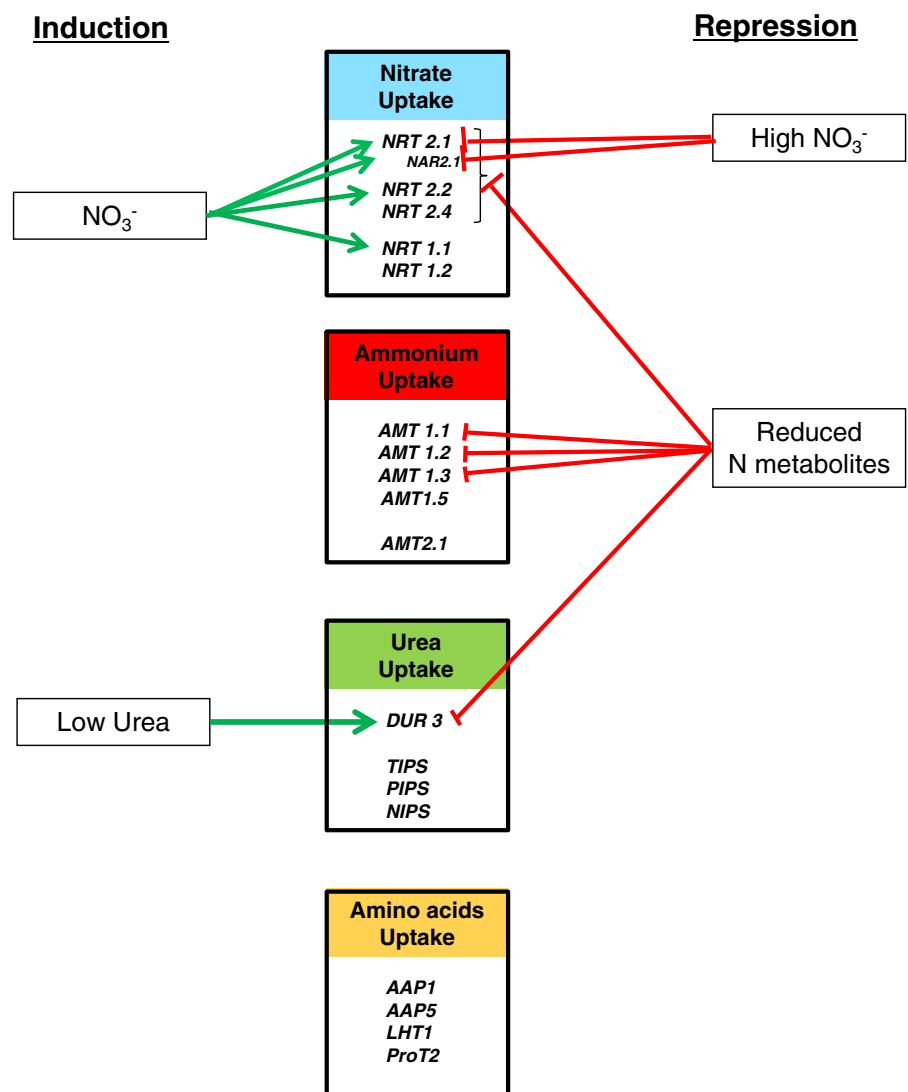
pathways governing feedback regulation of the NO_3^- HATS by the N status of the whole plant (Fig. 3). *HNI9/IWS1* came out of a genetic screen for mutants of *A. thaliana* impaired in the repression of the *pNRT2.1::LUC* reporter gene by high N supply (Girin et al. 2010). As compared to the non-mutagenized control *pNRT2.1::LUC* transformants, several of these mutants, named *hni* (*high nitrogen insensitive*), display a strongly increased expression of both the *pNRT2.1::LUC* reporter gene and the *NRT2.1* endogenous gene under repressive conditions (high N medium containing 10 mM NH_4NO_3). However, no difference was found for either gene between the mutants and the control line under permissive conditions (low N medium containing 0.3 mM NO_3^-), suggesting that *hni* mutations

specifically altered the mechanisms involved in the feedback repression of *NRT2.1* by high N status of the plant. Accordingly, downregulation of NO_3^- HATS activity by high N supply was also attenuated in the mutants (Girin et al. 2010). Positional cloning of one of the *HNI* genes (*NHI9*) revealed it encodes an IWS1 protein (Widiez et al. 2011). IWS1 proteins are evolutionary conserved components of the RNA polymerase II complex of transcription (Yoh et al. 2008). Unexpectedly, HNI9/IWS1-dependent repression of *NRT2.1* expression in response to high N supply correlates with marked changes in histone methylation (H3K27me3 marks) at the level of the chromatin associated with *NRT2.1* promoter (Widiez et al. 2011). This provides one of the first evidences that epigenetic
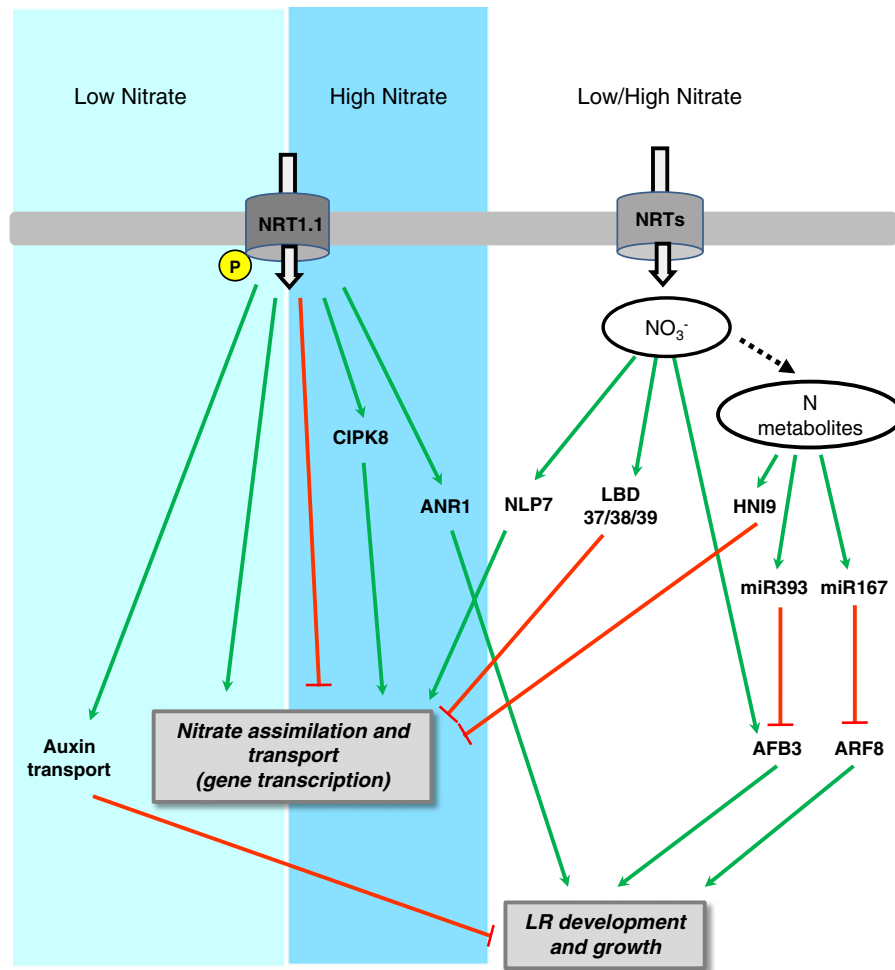
Fig. 3 Schematic representation of the N signalling pathways involved in the regulation of N-dependant gene expression and root development in *Arabidopsis thaliana*. The molecular components identified are shown. The dual-affinity NO_3^- transporter NRT1.1 has a dual role in NO_3^- dependant regulation of gene expression and control of root development. For NO_3^- concentrations under 0.5 mM (*light blue panel*) its phosphorylated form (T101 residue) modulates gene expression and represses the LR development by modulating auxin fluxes. Under NO_3^- concentration higher than 0.5 mM (*dark blue panel*) the non-phosphorylated form on NRT1-1 modulates gene expression through two distinct signalling pathways, including one dependant of the proteine kinase CIPK8. NRT1-1 is also involved in the root proliferation response in NO_3^- rich patches through the transcription factor ANR1. It is still unclear which form of NRT1-1 is involved in this specific response. NO_3^- itself independently of NRT1-1, modulates gene expression in two opposite ways involving the transcription factors NLP7 and LBD. Nitrate through its assimilation products is known to repress gene expression. However, the molecular mechanism remained unclear until the identification of an epigenetic mechanism dependant of HNI9, a key component of the RNA polymerase II complex of transcription. Indeed, HNI9-dependent repression of gene expression in response to high N supply correlates with marked changes in histone methylation of the chromatin. Nitrate and N metabolites affect root development through modulation of auxin perception (via AFB3) and auxin signalling (ARF8). *Green and red lines* indicate positive and negative effects, respectively

mechanisms may be triggered by nutrient signalling pathways, and participate in the adaptive responses of the plants to nutrient limitation.

In addition to this still very limited knowledge on the molecular mechanisms involved, the exact nature of the systemic signals of the whole plant N status remains elusive. Evidence supports the hypothesis that the satiety signal is related to the downward transport of reduced N metabolites. Amino acids are major constituents of both xylem and phloem saps, and it has been

suggested that the size and/or composition of the amino acid pool cycling between roots and shoot may integrate the N status of all organs, and convey this information to the roots (Cooper and Clarkson 1989). The use of tobacco and *A. thaliana* nitrate reductase deficient mutants has confirmed that products of NO_3^- assimilation are involved in the feedback repression of NO_3^- uptake (Gojon et al. 1998; Lejay et al. 1999). Furthermore, exogenous supply of amino acids, especially glutamine that is a major N storage form and accumulates under N satiety conditions, represses both NO_3^- uptake and the expression of key NO_3^- transporter genes in the roots (Muller and Touraine 1992; Krapp et al. 1998; Zhuo et al. 1999; Nazoa et al. 2003). In roots of *A. thaliana*, transcript accumulation of the N-repressible *AMT1.1* NH_4^+ transporter gene also displays a negative correlation with glutamine concentration in the tissues (Rawat et al. 1999). Despite circumstantial evidence clearly supports the view that amino acids (or at least some of them) are repressors of root NO_3^- and NH_4^+ uptake systems, this hypothesis still awaits final confirmation because the underlying mechanisms remain obscure at the molecular level. Furthermore, not all data are consistent with a negative correlation between downward phloem transport of amino acids and NO_3^- uptake rate in the roots (Tillard et al. 1998; Lainé et al. 1995). Therefore, alternative hypotheses may also be considered. For instance, systemic signalling of P or S status of the plant has unexpectedly been shown to rely on shoot-to-root transport of miRNAs (Chiou et al. 2006; Pant et al. 2008; Liu et al. 2009; Kawashima et al. 2009). It is not known yet whether long-distance transport of specific miRs is also associated with changes in N status of the whole plant, or not. However, the occurrence of N-regulated miRs has been recently reported (Pant et al. 2009; Gifford et al. 2008; Vidal et al. 2010; Zhao et al. 2011), opening new perspectives for the search of N-related signal molecules.

Finally, phytohormones and more precisely cytokinins (CKs) have been proposed to coordinate demand and acquisition of N (Kiba et al. 2011). In *A.thaliana*, a positive correlation between plant cytokinin contents and nitrate concentration in the growth medium as also been reported implying that CKs are also a N status signal (Kiba et al. 2011). At the molecular level, early steps of cytokinins biosynthesis is mediated by genes encoding adenosine phosphate-isopentenyltransferase (*IPT* genes). In *A. thaliana* IPT is encoded by seven genes and among them, the NO_3^- inducible *IPT3*

plays a crucial role (Takei et al. 2004). Given that *AtIPT3* is expressed in phloem, it is likely that CKs synthesized by AtIPT3 in shoots function as a shoot-to-root long-distance signal of shoot NO_3^- availability (Kiba et al. 2011). In line with these results, it was found from both microarray analysis (Brenner et al. 2005) and quantitative PCR (Kiba et al. 2011) that CKs repress high-affinity root NO_3^- transporter genes in *A.thaliana* strengthening the hypothesis that CKs act as a N satiety signal to inhibit NO_3^- uptake in the root (Fig. 4). Recently, based on split root experiments, Ruffel et al. (2011) confirmed that CKs plays a major role in the root-shoot-root signalling. Interestingly, it has been reported that CKs negatively regulate other nutrient acquisition-related genes in *A. thaliana*, such as high-affinity phosphate transporter genes, high-affinity sulphate transporter genes or iron deprivation inducible genes. However, the underlying mechanisms coordinating the wide range of CK-dependent morphological and physiological response is not yet known.

One intriguing question related to the systemic regulation of N intake into the plant is to know whether the signalling pathways are common for the acquisition of the various N sources (NO_3^-, NH_4^+, urea…). Although it may appear likely that some kind of common mechanism should be involved in the integrated control of root N uptake, whatever the N form, evidence for this is still lacking. Rather, several studies pointed out that the mechanisms ensuring the feedback repression of *NTR2* and *AMT1* transporter genes are different (Gansel et al. 2001; Krouk et al. 2006; Widiez et al. 2011). For instance, Gansel et al. (2001) proposed that NH_4^+ uptake was predominantly regulated by the local N status of the roots rather than by the N status of the whole plant. Furthermore, the overall transcriptome responses to N deficiency or N treatments are predominantly specific of the N form supplied to the plant (Ruffel et al. 2008; Merigout et al. 2008). Therefore, it is not known how a plant under mixed N nutrition can coordinate concurrent uptake of different N sources to tune its overall N acquisition to its N demand for growth.

Besides organic N metabolites, there are indications that NH_4^+ itself may also act as a repressor of root N uptake systems. Indeed, the use of methionine sulfoximine (MSX), a potent inhibitor of glutamine synthetase that ensures primary assimilation of NH_4^+ into glutamine, failed to alleviate repression of *NRT2.1* expression by high N supply in *A. thaliana* (Zhuo et al.

Fig. 4 Schematic representation of the action of phytohormones in the NO_3^- regulation of root and shoot growth, and of NO_3^- uptake in *Arabidopsis thaliana*. *Brown and green boxes schematise* respectively root system and shoots. Molecular players identified are listed in the *white box*. *Green, red and black lines* indicate positive, negative and complex effects, respectively. *Plain and dashed lines* represent known or putative functional links. *Question marks* represent unconfirmed results
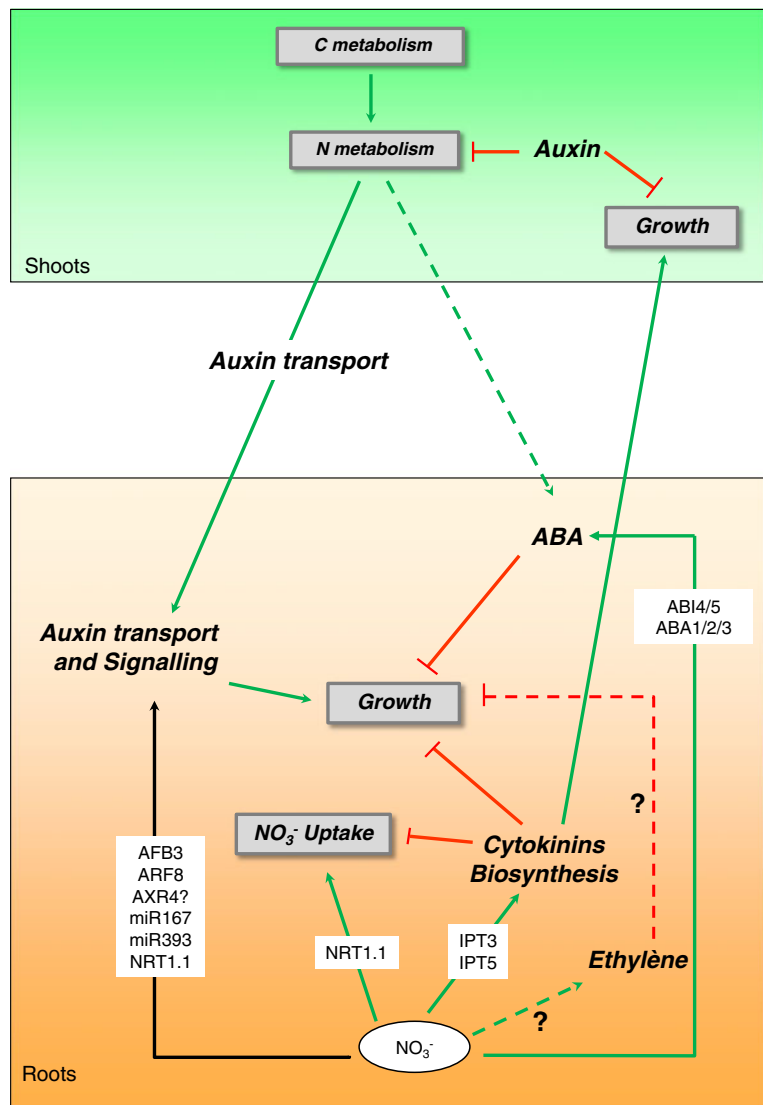
1999). Finally, although induction appears to be a prominent response of NO_3^- transporter genes to NO_3^-, some are at the opposite strongly repressed by NO_3^- supply (e.g., *NRT2.5* of *A. thaliana*, Okamoto et al. 2003), or surprisingly subject to a dual regulation (induction/repression) by NO_3^-, like *NRT2.1* of *A. thaliana* (Fig. 2). Indeed, despite *NRT2.1* expression is upregulated by short-term NO_3^- supply (see above), it is also strongly downregulated after provision of high NO_3^- concentration for several days (Muños et al. 2004; Krouk et al. 2006). Such a time course, i.e., initial induction by NO_3^- followed by subsequent repression was also reported for root NO_3^- uptake in NR-deficient barley plants, supporting the hypothesis that NO_3^- *per se* is the signal responsible for both phases of the response (King et al. 1993). Interestingly, NRT1.1 that triggers *NRT2.1* induction by NO_3^- is also required for its downregulation by long-term high NO_3^- supply (Fig. 3, Muños et al. 2004; Krouk et al. 2006). Thus, unlike wildtype plants, *nrt1.1* mutants keep a high level of both NRT2.1 mRNA accumulation and NO_3^- HATS activity under ample NO_3^- provision. A surprising observation is that *NRT2.1* overexpression in *nrt1.1* mutants is also found in plants fed with high concentrations of both NO_3^- and NH_4^+ (Muños et al. 2004; Krouk et al. 2006). This suggests that NRT1.1 might

also participate in the feedback downregulation of *NRT2.1* by reduced N metabolites, or that *NRT2.1* is repressed only when both downregulatory mechanisms, by NO_3^- and reduced N metabolites respectively, are active. There are two findings supporting the latter hypothesis. First, unlike feedback regulation by N status of the plant, NRT1.1-dependent repression of *NRT2.1* by high NO_3^- is a local regulation, only active in the roots that are in contact with high NO_3^- concentration (Krouk et al. 2006). Second, repression of *NRT2.1* expression by high NH_4NO_3 supply (e.g., 10 mM) is gradually alleviated when NO_3^- concentration is decreased to a low level (e.g., <0.5 mM), while NH_4^+ concentration remains at 10 mM (Krouk et al. 2006). The mechanism by which NRT1.1 represses *NRT2.1* expression in presence of high NO_3^- concentration is unknown. However, Rubin et al. (2009) identified three transcription factor genes (*LBD37/38/39*) that are strongly induced by NO_3^-, and act as repressors of *NRT2.1* transcription (Fig. 3). Although no direct connection has been made to date between NRT1.1 and regulation of *LBD37/38/39*, it will certainly be worth investigating whether NRT1.1-dependent repression of *NRT2.1* requires one or several of these transcription factors.

Post-transcriptional regulation of root N transporters

In addition to the various mechanisms detailed above that act at the gene expression level, increasing evidence indicates that post-transcriptional regulation of root N transporters also plays a key role in the control of the uptake function by the roots. However, studies on this point are scarce and detailed information is only available for NRT1.1 and NRT2.1 NO_3^- transporters, and AMT1 NH_4^+ transporters of *A. thaliana*.

As mentioned previously, NRT1.1 can be phosphorylated at the T101 residue, and experiments in both *Xenopus* oocytes and plants have suggested that the phosphorylated form is a high-affinity transporter, whereas the non-phosphorylated form is a low-affinity transporter (Liu and Tsay 2003; Ho et al. 2009). Interestingly, phosphorylation of NRT1.1 is promoted by low NO_3^- availability (Liu and Tsay 2003; Ho et al. 2009), illustrating a mean by which functional characteristics of a transporter are modulated to improve transport efficiency as a function of the actual availability of its substrate in the external medium.

The occurrence of post-transcriptional regulation of AMT1.1 was shown by the fact that its ectopic expression in both tobacco and *A. thaliana* transgenic plants failed to prevent changes in its transcript accumulation in response to N starvation, indicating N-dependent regulation of *AMT1.1* mRNA stability (Yuan et al. 2007). However, the best characterized mechanism for posttranscriptional regulation of AMT1 transporters is the so-called allosteric feedback inhibition that has recently been identified in *A. thaliana* (Loqué et al. 2007, Neuhauser et al. 2007). The AMT1.1 transport system is a trimer that can be shut off when one or several subunits are phosphorylated on the T460 residue (Lanquar et al. 2009). This phosphorylation leads to a conformational modification of the cytosolic C terminus domain that trans-inhibits the other subunits. It is specifically triggered by external NH_4^+ in a time and concentration manner, thus defining a local autoregulatory mechanism restricting uptake.

Little is known about posttranscriptional regulations of NRT2 transporters. Nevertheless, the importance of such a regulation has been supported by data obtained in tobacco (Fraisier et al. 2000), barley (Ishikawa et al. 2009) and *A. thaliana* (Wirth et al. 2007; Laugier et al. 2012). In the later species, ectopic expression of *NRT2.1* actually resulted in a high and almost constant mRNA level in the roots, which did not prevent downregulation of NO_3^- HATS activity by high N supply. Depending on the conditions, downregulation of the HATS was associated with decreased levels of both NRT2.1 and NAR2.1 proteins abundance in membranes, or not (Laugier et al. 2012). This pinpoints the occurrence of multiple levels of NO_3^- HATS regulation, including posttranslational control of NRT2.1/NAR2.1 activity. Interestingly, NRT2.1 has recently been shown to be phosphorylated in response to NO_3^- supply (Engelsberger and Schulze 2012). However, the functional significance of this posttranslational modification remains to be determined.

Regulation of root system architecture

Size and architecture of the root system are major factors of nutrient acquisition efficiency because they determine the total volume of soil explored by the plant, and the total surface of exchange between roots and soil solution. Accordingly, many nutrients have a dramatic impact on root growth and development, and the resulting changes in root system architecture

(RSA) generally play an important role in the plant adaptation to fluctuations of external nutrient availability. RSA of many species is strongly dependent on both external N availability and internal N status of the plant. RSA modifications have been reported to be particularly important in response to nutrients with low mobility in soil, such as Pi or NH_4^+. However, NO_3^- is also well known to markedly affect root development and growth, despite its high mobility (Zhang and Forde 2000; Gojon et al. 2009). This might reflect a crucial role of RSA for competing with other plants and soil microorganisms even for nutrients that can move almost freely in the soil. Interestingly, in species with a pivotal root system, N availability strongly modulates root branching, whereas it has a limited impact on primary root growth (Zhang and Forde 2000, Linkor et al. 2002; Lima et al. 2010; Chapman et al. 2011). Besides this general trend, defining a typical RSA phenotypic response to changes in N availability is almost impossible, as it is highly depending of the morphology of the root system, the species and the other abiotic environmental conditions (ie water availability, pH, other nutrients…). However two major aspects of response appear to be common to many species: (i) a systemic repression of lateral root growth by high N status, and (ii) a local stimulation of lateral root growth by exogenous NO_3^- or NH_4^+ supply.

Repression of root growth by high N status of the plant

High N supply represses root growth in many species. In *A. thaliana*, NO_3^- concentration over 10 mM inhibits lateral root (LR) development, by preventing meristem activation in newly emerged primordia (Fig. 5). This leads to an accumulation of short LRs blocked just after emergence from the primary root (Zhang et al. 1999). This effect is reversible as transferring seedlings to NO_3^--limiting media results in a release of LR inhibition within 24 h. In maize, high NO_3^- availability (5 mM) strongly inhibits not only LR elongation but also growth of primary, seminal and crown roots (Chun et al. 2005, Tian et al. 2008). In *A. thaliana*, the repression of LR growth by high NO_3^- is systemic because it can be observed in split root plants where high NO_3^- supply on one side of the root system is able to repress LR elongation in the other portion of the root system fed with a low NO_3^- medium (Zhang et al. 1999). The nature of the signal

responsible for the repression is unclear. A high shoot NO_3^- status seems important for the inhibitory response as this effect is enhanced in a nitrate reductase deficient mutant, suggesting that systemic signalling of NO_3^- accumulation in the shoot causes LR inhibition (Zhang et al. 1999). However, NH_4^+ and organic N molecules such as glutamine also trigger inhibition of LR growth. Gifford et al. (2008) reported that glutamine was the predominant signal regulating repression of LR emergence. Indeed, they observed that, in *A. thaliana*, the LR growth inhibition induced by high NO_3^- concentration can be alleviated by MSX, and restored when glutamine is added in the culture medium. Altogether, these data might suggest that there are at least two different signals regulating different stages of LR growth, with the "glutamine-dependent signalling pathway" controlling LR initiation/pre-emergence vs. the "NO_3^--dependent signalling pathway" controlling LR elongation. Furthermore, several lines of evidence suggest the implication of phytohormones as long distance signals involved in the N regulation of root development and growth (Fig. 4). Forde (2002) hypothesized that NO_3^- accumulation in shoots may inhibit the flux of auxin down to the roots, leading to a failure of the lateral roots to pass an auxin-requiring checkpoint in LR development. In agreement with this hypothesis, it was measured that transferring seedlings from high to low NO_3^- increases Indol Acetic Acid (IAA) root content by 50 % within 24 h (Walch-Liu et al. 2005), and that soybean seedlings grown on 1 mM NO_3^- have four-fold higher IAA concentration than plants grown on 8 mM NO_3^- (Caba et al. 2000). Furthermore, inhibition of maize root growth by high NO_3^- levels has been closely related to a reduction level of IAA in roots, especially in the section close to the root tip where IAA content can be reduced up to 80 % (Tian et al. 2008). In agreement, these authors found that exogenous IAA and NAA (a synthetic analogue of auxin) are able to restore growth in high NO_3^- conditions. There is also evidence that a second plant hormone, abscissic acid (ABA) may be involved in the systemic regulation of root growth. Three ABA-insensitive mutants (*abi4.1*, *abi4.2* and *abi5.1*) and four ABA synthesis mutants (*aba1.1*, *aba2.3*, *aba2.4* and *aba3.2*) display a significantly reduced inhibition of LR growth under abundant NO_3^- supply (Signora et al. 2001). Furthermore, *LR ABA insensitive* (*labi*) mutants, that can still produce LRs in the presence of

Fig. 5 Schematic representation of the effects of NO_3^- on lateral root initiation and growth in *Arabidopsis thaliana*. The central part of the figure represents the different developmental stages of lateral roots from initiation (*chart most to the left*) to elongating lateral roots (*chart most to the right*). The upper part represents the effect of NO_3^- whereas the lower represent the NRT1-1 dependent responses to low NO_3^- concentration (*light blue*) or local nitrate supply (*dark blue*). *Green Yellow and Pink pannels schematise* respectively Auxin, ABA signalling pathways. *Black boxes* are the different phytohormones identified. *Purple dot, blue arrows and brown arrowheads* represent auxin accumulation, auxin flux toward primary root tissues and lateral roots elongation rate, respectively. *Green and red lines* indicate positive and negative effects, respectively
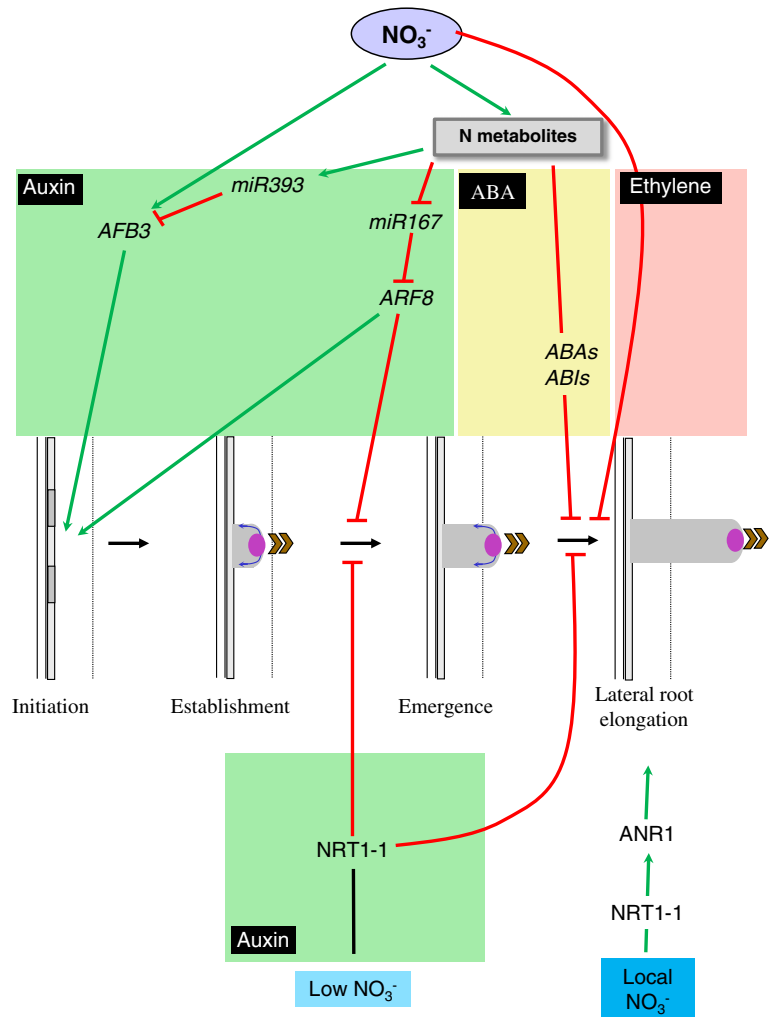
ABA, are also less sensitive to high NO_3^- (Zhang et al. 2007), and exogenous ABA supply mimics the NO_3^--induced inhibition of LR elongation (De Smet et al. 2003) although this seems to be a little later than the arrest observed in the presence of high NO_3^-. In addition, these groups demonstrated that exogenous auxin supply does not overcome the ABA-induced arrest, suggesting that ABA and auxin are acting through independent pathways. Cytokinins were also proposed to participate to root growth limitation in maize plants supplied with high NO_3^- (Tian et al. 2005). Recently, ethylene was shown to be involved in high NO_3^- LR growth limitation in *A. thaliana*. Indeed, transferring plants from low to high NO_3^- induces a rapid burst of ethylene production, leading to root growth limitation. In agreement, this root growth inhibition is suppressed in ethylene signalling

mutants or when ethylene synthesis antagonists are present in the culture medium (Tian et al. 2009). However the precise role of both cytokinins and ethylene remain elusive.

Stimulation of lateral root growth by NO_3^- and NH_4^+

Local N availability also directly affects root growth. The most documented response concerns NO_3^- but several reports also pinpoint a similar effect of NH_4^+ whereas very little is known about local organic N supply. A major illustration of the effect of local N concentration which has been well documented in the literature for many years is the so-called "root proliferation response", i.e., the ability of plants to proliferate roots preferentially in nutrient-rich patches (Nobbe F. 1862). Drew (1975), through a detailed RSA

analysisdemonstrated that a nutrient rich 'patch' could elicit a localized increase in LR initiation and elongation in barley. However, the importance of root proliferation for N capture from patches appeared a paradox because, although root proliferation within N-rich patches could easily be demonstrated, the benefit in terms of N capture from the patch could not (van Vuuren et al. 1996; Hodge et al. 1998; Fransen et al. 1998). These three studies all used different plant species but in all cases plants were grown as individuals. When considered within the context of inter-plant competition, it has been demonstrated that when plants are competing for a common patch of organic N, root proliferation does make sense: the species which proliferated the most captured the most N (Hodge et al. 1999, 2000; Robinson et al. 1999).

In contrast with its systemic inhibitory effect on LR growth, NO_3^- locally promotes initiation and/or elongation of LRs (Fig. 5). This adaptive response was extensively studied in *A. thaliana*, in which Zhang and Forde (1998) demonstrated that a localised patch of NO_3^- in a split agar plate elicited a preferential LR growth in the region of the patch. This resulted from the combination of a strong increase in LR elongation and a marked stimulation of LR primordia emergence in the NO_3^--enriched region (Zhang and Forde 1998; Zhang et al. 1999; Linkohr et al. 2002; Remans et al. 2006; Krouk et al. 2010b). NO_3^- rather than its downstream metabolites appears to be the signal for the stimulation as a mutant in nitrate reductase shows the wildtype response (Zhang and Forde 1998). Besides this positive effect of local NO_3^- concentration, Linkohr et al. (2002) found that within the same plant, the unequal distribution of LRs between NO_3^--rich and NO_3^--poor patches is also due to repression of lateral root elongation in the NO_3^--poor patch, which was recently confirmed by Krouk et al. (2010b). Finally, if the localized NO_3^- supply is made on a too restricted portion of the total root system, the consequent N limitation alleviates the systemic repression by high N status, further amplifying the local proliferation response. Several molecular components of the signalling pathway responsible for the local positive effect of NO_3^- have now been identified in *Arabidopsis* (Fig. 5). The ANR1 MADS box putative transcription factor was characterized as a critical component for the local root elongation stimulation as the response is drastically reduced in *ANR1* antisens or co-suppressed plants (Zhang and Forde 1998) and

is stimulated in *ANR1* inducible transgenic lines (Gan et al. 2012). Zhang and Forde (1998) also speculate that a downstream target of local NO_3^- response might be *AXR4* gene as they reported that the *axr4* mutant showed attenuated response to local NO_3^- supply (Zhang et al. 1999). However, Linkohr et al. (2002) found in similar experiments that the *axr4* response is similar to WT. More recently, the *Arabidopsis* NO_3^- transporter NRT1.1 was reported to also play a crucial role in the signalling process (Fig. 5), possibly as an upstream membrane NO_3^- sensor responsible for the initial perception of the external NO_3^- concentration that triggers the whole regulatory pathway (Remans et al. 2006). Indeed, these authors found that *nrt1.1* mutants display a strongly reduced response to a localised NO_3^- supply, similarly to *ANR1* downregulated lines. Interestingly, the reduced responsiveness was not due to a decrease in NO_3^- acquisition as the specific uptake activity was not reduced (Remans et al. 2006). More importantly, *chl1* mutants have a markedly reduced abundance of ANR1 transcript, suggesting that NRT1.1 may act upstream ANR1. These data further support the hypothesis that NRT1.1 acts as a NO_3^- transceptor (Gojon et al. 2011, see above). Interestingly, unlike for the other NRT1.1-dependent signalling pathways, a precise molecular mechanism has been proposed to account for the role of NRT1.1 in the NO_3^- regulation of development of the LR primordia (Krouk et al. 2010b). Indeed, the NO_3^--induced stimulation of LR emergence and growth is associated with an increased accumulation of auxin at the apex of primordia or of newly emerged LRs. This auxin maximum is mandatory for further development and growth of these organs (Benkova et al. 2003). NRT1.1 is required for the local increase of auxin accumulation in response to NO_3^-, not because it promotes this accumulation in presence of NO_3^-, but because it prevents it in the absence of NO_3^- (Krouk et al. 2010b). Accordingly, primordia and newly emerged lateral roots of *chl1* mutants display a constitutively high level of auxin, which results in a constitutive high rate of primordia emergence and growth, regardless of the external NO_3^- concentration (Krouk et al. 2010b). The reason why NRT1.1 controls the NO_3^--induced accumulation of auxin in lateral roots is that this protein is able to transport auxin in a NO_3^--dependent manner, as shown in heterologous expression experiments in *Xenopus* oocytes, *S. cerevisiae* or BY2 cells (Krouk et al. 2010b).

Altogether, these data led to the proposal of a model postulating that in the absence, or at low external availability, of NO_3^-, NRT1.1 behaves as an auxin transport facilitator that contributes to remobilize the hormone out of the lateral root toward the parent root, therefore repressing emergence and growth of this organ. At high NO_3^- concentration, auxin transport facilitation by NRT1.1 is inhibited, and auxin accumulates at the apex of the lateral root, which in turn promotes emergence and growth (Krouk et al. 2010b). Such mechanism has only been identified in *A. thaliana.* However, recent findings suggest that a protein belonging to the NRT1 (PTR) family, encoded by the *M. truncatula LATD* gene regulates the legume root system architecture. Indeed, *LATD* is expressed in the root meristem and elongation zone (Yendrek et al. 2010), and is required for the maintenance of primary and LR growth and of symbiotic nodule meristem. In addition, *latd* mutants are insensitive to the inhibition of the primary root growth by NO_3^- (Yendrek et al. 2010). Besides this, another member of the *M. truncatula* PTR family, MtNRT1.3, has been characterised (Morere-Le Paven et al. 2011). Like the *A.thaliana* NRT1.1, this transporter behaves as a dual affinity NO_3^- transporter when expressed in *Xenopus* oocytes. Interestingly, it has been associated with a QTL of root development, although the demonstration that *MtNRT1.3* is the QTL still remains to be done. Taken together, these results suggests that the NO_3^- sensing and signalling function of PTR members is not only restricted to *A. thaliana* but may also be observed in other species.

An additional evidence indicating that auxin signalling is involved in the local stimulation of LR growth by NO_3^- is the recent finding that the gene encoding the AFB3 auxin receptor is strongly induced by NO_3^-, and that stimulation of LR primordia initiation by NO_3^- is markedly reduced in an *afb3* mutant (Vidal et al. 2010).

The hypothesis that, in addition to NO_3^-, external NH_4^+ could also be sensed specifically by the roots, and could trigger adaptive RSA responses has been a matter of debate. Heterogeneous NH_4^+ supply to sand-grown barley plants (Drew 1975) or to *Cedrus atlantica* (Boukcim et al. 2006) also resulted in a localized proliferation of LRs. Split-root experiments in *A. thaliana* indicated that the localized stimulation of LR elongation induced by NO_3^- could not be mimicked by NH_4^+ (Zhang et al. 1999; Remans et al. 2006). Nevertheless, recent reports provide convincing evidence that NH_4^+ present in the external medium is indeed perceived as a signal molecule by the roots, possibly via AMT1 transporters (see above and Lanquar et al. 2009), and can regulate LR development. Indeed, a detailed reconsideration of the action of NH_4^+ on RSA in *A. thaliana* unravelled specific effects different from those of NO_3^-. Unlike NO_3^- that predominantly promotes LR emergence and elongation, localized NH_4^+ supply was found to mainly increase initiation of second and third order LR primordia, leading to a highly branched root system, while further elongation of pre-emerged root initials was strongly reduced, resulting in a much more "bushy" architecture than with NO_3^- (Lima et al. 2010). Taken together, these results suggest that NO_3^- and NH_4^+ have complementary actions on RSA, that were shown to be at least partly additive when NH_4^+ and NO_3^- are supplied together (Lima et al. 2010). Interestingly, the RSA responses to localized NH_4^+ supply cannot be explained by a nutritional effect alone and required the presence of a functional AMT1.3 transporter (Lima et al. 2010), thus making an interesting parallel with the NRT1.1-dependent lateral root proliferation in response to localized NO_3^- supply.

Very little is known about the RSA response to local organic N application. However, it has been reported that L-glutamate is able to inhibit primary root growth even at low concentration (0.05–0.5 mM) (Filleur et al. 2005 and Walch-Liu et al. 2006). This response was highly specific for L-glutamate, as similar concentrations of related amino acids or of D-glutamate had no effect. The effects on RSA are complex because although inhibition of primary root growth was detectable within 24 h of transfer to L-glutamate, LRs only acquired sensitivity after some time (once they reach an average length of 5–7 mm). Finally, L-glutamate promoted an outgrowth of LRs close to the root tip. The net result is a bushy and more branched root system similar to the phenotype seen when *A. thaliana* seedlings were grown on a limiting supply of P (Williamson et al. 2001, Lopez Bucio et al. 2002; Svistoonoff et al. 2007). This response is not only observed in *A. thaliana* but also in *Arabidospis*-related species, tomato and poppy (Walch-Liu et al. 2005). Interestingly the inhibitory effect of L-Glutamate is alleviated by NO_3^-, through the involvement of a NRT1.1-dependent signaling pathway (Walch-Liu and Forde 2008).

Slowing down of shoot growth

Most often, it is assumed that the detrimental effects of sub-optimal N availability on plant biomass production and yield are due to metabolic limitations in the various organs, resulting from inadequate N acquisition by the root system. In other words, the plant does not reach its full growth potential because internal N is not available enough to ensure synthesis of biomolecules at the level required to sustain unlimited growth. This is particularly crucial in the shoot, because photosynthesis is a high N-demanding process as Rubisco alone can account for up to 20–40 % of total N in the leaves (Evans 1989). However, there is now mounting evidence that slowing down of shoot growth under situations of N limitation is not only a consequence of internal N deficiency, but also a fast adaptive response of the plant to prevent internal N deficiency. Indeed, numerous studies have shown that reduction of leaf expansion rate occurs very fast in response to N limitation or starvation, and precedes any measurable symptom of organic N deficiency (Chapin et al. 1988; Chapin 1991; Palmer et al. 1996; Rahayu et al. 2005). Furthermore, it is known for long that N-limited plants accumulate large amounts of carbohydrates, indicating that decay of photosynthesis is a consequence rather than a cause of growth rate restriction (Radin and Eidenbock 1986; Rufty et al. 1988; Chapin 1991). The concept that fast slowing down of growth constitutes an adaptive response to N limitation is not new, as it has been proposed to be part of a centralized system of physiological responses to nutrient or water stresses (Chapin 1991). The role of this centralized system is to improve survivorship under unfavourable conditions, by restraining growth and thus preventing metabolic disorders associated with restricted nutrient acquisition. Most probably, fast slowing down of shoot growth also contributes to a modified allocation of assimilates in favour of the root system (less demanding for N), leading to the well kown decrease of the shoot to root biomass ratio in response to N limitation (Hermans et al. 2006). Thus, concurrently with regulatory mechanisms aiming at maintaining the N offer to the various organs through improved N acquisition efficiency (see above sections), it clearly appears that plants ensure N homeostasis by also reducing the N demand through reduced growth of the above ground organs.

Compelling evidence has accumulated supporting the hypothesis that the regulatory mechanisms quickly modulating shoot growth as a function of N availability also involve a tight connection between N and hormone signalling pathways, as it is the case with the N regulation of root system architecture (Chapin 1991; Sakakibara et al. 2006; Rubio et al. 2009; Vidal et al. 2010; Krouk et al. 2011). As conceptualized by Chapin (1991), changes in external N availability may trigger fast responses of shoot growth through modified absissic acid (ABA) and/or cytokinin (CK) levels. Interestingly, recent transcriptomic studies indeed indicate that many NO_3^--responsive genes related to primary or energy metabolism and to photosynthesis are also regulated by ABA and/or cytokinins (Nero et al. 2009). Despite some indication that decreased N supply affects ABA accumulation in shoots (Chapin 1991; Brewitz et al. 1995), correlation between leaf ABA levels and short- or long-term N-induced changes in shoot growth was found to be relatively poor (Rahayu et al. 2005). However, the recent finding that the *A. thaliana* NRT1.2 transporter is also able to transport ABA may provide some new hypotheses on this point (Kanno et al. 2012). In contrast, the connection between N and CK signalling, and its role in the regulation of shoot growth is much better characterized (Sakakibara et al. 2006). N provision to the plant results in many different species in an increase in CK content of the tissues (Samuelson and Larsson 1993; Wagner and Beck 1993; Takei et al. 2001, 2004). Most importantly, this also leads to a strong stimulation of CK transport from root to shoot in the xylem (Takei et al. 2001, 2004). In *A. thaliana*, this is due to the specific induction by N compounds of a set of key enzymes involved in CK biosynthesis in the roots, namely IPTs and cytochrome P450 monooxygenases (CYP735A), that promote trans-zeatin (an highly active CK form) production and export (also as trans-zeatin riboside) into the xylem (Sakakibara et al. 2006). Among the 7 *IPT* genes present in the *A. thaliana* genome, *IPT3* and *IPT5* were found to be induced in the roots by N supply, with *IPT3* responding specifically to NO_3^- within only 1 h after treatment (Miyawaki et al. 2004; Takei et al. 2004; Wang et al. 2004). Accordingly, NO_3^--induced CK biosynthesis was strongly reduced in an insertion mutant of *IPT3* (Takei et al. 2004). In both *A. thaliana* and maize, stimulation of CK xylem transport by N supply activates CK signalling cascades by His-Asp phospho-relay in leaves (Sakakibara et al. 1998; Taniguchi et al. 1998). Since CK promote cell division in the shoot apical meristems, this suggests that these hormones act as long-distance

signals of N availability in the roots to directly and rapidly stimulate shoot growth (Fig. 4). Accordingly, changes in leaf expansion rate in response to NO_3^- in tomato (that can be recorded as fast as 4 h after a change in NO_3^- supply) are strongly correlated with changes in the zeatin and zeatin riboside concentrations in the xylem sap (Rahayu et al. 2005). Moreover, the observation that some of the molecular components involved in this system respond specifically to NO_3^- and not to other N compounds (i.e., specific induction of *IPT3* by NO_3^-) likely accounts for the predominant effect of NO_3^- in stimulating shoot growth. Indeed, increase in leaf expansion rate after NO_3^- supply has been recorded both in plants previously N-starved or precultured with NH_4^+ as an N source (Walch-Liu et al. 2005; Rahayu et al. 2005). Thus, at least part of the growth difference observed in many plant species between NO_3^- or NH_4^+ nutrition may not be due to NH_4^+ toxicity *per se*, but to the fact that only NO_3^- is able to fully activate the CK signalling cascade stimulating shoot growth. This indicates a direct link between CK signalling in shoots and NO_3^- sensing systems in roots that is further documented by the report that induction of *ITP3* by NO_3^- is partly dependent on the NRT1.1 NO_3^- transceptor in *A. thaliana* (Kiba et al. 2011). Although it is difficult to extrapolate from one single example (connection between NRT1.1 and CK signalling), the model that emerges from these observations is that plants are able to quickly modulate (within hours) their shoot growth as a function of their perception of nutrient availability to the roots. This certainly allows fast survival responses as soon as the environment is detected to be unfavourable for growth, and thus before any decrease in the whole plant nutrient status had occurred.

If correct, this model implies that manipulation of the nutrient sensing systems in the roots will affect the response of shoot growth to changes in nutrient availability. Interestingly, the NRT1.1 NO_3^- transceptor, which participates in the NO_3^- regulation of LR growth in *A. thaliana* (see above section), has recently been shown to also modulate shoot growth as a function of the N source (Hachiya et al. 2011). However, there is no evidence yet that a putative role of NRT1.1 in the regulation of shoot growth is due to modified root-to-shoot CK signalling. Another intriguing report in this general context is the recent paper by Rouached et al. (2011) concerning the PHO1 phosphate (Pi) transporter of *A. thaliana*. PHO1 is predominantly expressed in the root vasculature, and is believed to play a key role in the

translocation of Pi to the shoot via the xylem (Poirier et al. 1991; Hamburger et al. 2002). Accordingly, null *pho1* mutants grown in soil display a dramatic reduction of Pi export from the roots, leading in the shoot to a markedly diminished Pi content, a strong reduction of growth, and a gene expression profile typical of P deficiency. Unexpectedly, *PHO1* underexpressors or transformants expressing the rice *PHO1* ortholog in a *pho1 A. thaliana* mutant background, show almost complete recovery of shoot growth and suppression of transcriptome responses triggered by P deficiency, despite an only very partial complementation of the Pi export function (Pi translocation rate 2.6 to 4.2-fold lower than in the wild type, Rouached et al. 2011). As a consequence, these lines have very low shoot Pi content, similar to that of *pho1* mutants (i.e., 3 to 4-fold lower than the wild type). Altogether, these results indicate that the responses of the above ground organs to Pi deficiency (restricted growth and transcriptome reprogramming) can be uncoupled from low Pi content of the shoot, but require a fully functional PHO1 transporter in the roots. Although this does not demonstrates that PHO1 has an actual Pi sensing role, it will certainly be worth investigating why its genetic manipulation yields the exciting phenotype of a fully normal shoot growth with only one-third of the Pi content of wild type shoots.

Conclusion and future prospects

There are at least three aspects for which our understanding of the mechanisms of plant adaptation to changes in N availability has significantly progressed within the past years. First, the molecular structure of both NO_3^- and NH_4^+ root uptake systems has been further detailed to reach an almost comprehensive knowledge. This allowed identifying the key transporters targeted by the N regulatory mechanisms, and responsible for the functional flexibility of root N acquisition. Second, major advance has been made in the elucidation of the N signalling pathways involved in sensing of external N availability. This is most obvious in the case of NO_3^-, with the mounting hypothesis that some of the membrane transporters of the NRT1 family actually fulfil a dual transport/sensing function, and act as transceptors. Finally, a strong body of evidence indicate that many adaptive developmental responses to changes in N availability are triggered by a direct connection between N and

hormone signalling pathways. In some instances, the two later aspects strongly interact, as highlighted by the recent finding that both NRT1.1 and NRT1.2 transporters of *A. thaliana* have a dual NO_3^-/hormone substrate specificity. Yet, a lot remains to be done in this field. Concerning the structure and regulation of the root uptake systems, it remains to determine whether the conceptual model we now have in model plants such as *A. thaliana* (i.e., functional organisation of NRT1, NRT2, AMT1, DUR3 transporter familles) is also valid for crops, or not. Furthermore, if it appears now feasible to almost totally suppress root N uptake capacity of a plant by knockingdown the few relevant transporter genes, attempts to improve root N uptake efficiency by manipulating these transporters has been hardly successful to date. Clearly, there are either other limiting steps or levels of regulation and integration we still do not understand, and which prevent a simple genetic or biotechnological use of the key transporter genes. Deciphering the N sensing and signalling pathways that allow the plant to determine the amount and precise localization of the N resources in the soil is certainly a major priority. This is important to improve our basic knowledge of biological processes that are most propably specific to plants, but this is also important because it now appears that these sensing and signalling pathways are directly connected to the control of growth through hormone signalling. Thus, by manipulating the N sensing systems, one can expect improving crop yield at low N fertilizer input by preventing the slowing down of growth directly triggered by the perception of a suboptimal level of N in the soil. Another area where a significant breakthrough is needed relates to whole plant N signalling. Although extensively described at the physiological level, the molecular mechanisms responsible for the systemic signalling of plant N status remain almost totally unknown. This strongly departs from major advance made on P or S signalling, and raises questions about the relevant strategies to develop to reach this goal. Finally, the dramatic plasticity of RSA in response to N availability has been only recently fully acknowledged. The mechanisms responsible for it have begun to be unravelled, yielding original and exciting hypotheses. No doubt that exploiting this plasticity of root development will be an important strategy to improve N use efficiency in plants (Lynch 2007; Lynch and Brown 2012; Pages 2011). This certainly calls for major efforts toward high-throughput phenotying of RSA, in particular in the field.

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