Nitrogen control of developmental phase transitions in Arabidopsis thaliana

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Abstract

Nitrogen (N) is an essential macronutrient and a key structural component of macromolecules in plants. N nutrients and metabolites can act as signals that impact on many aspects of plant biology. The plant life cycle involves a series of developmental phase transitions that must be tightly coordinated to external and internal cues in order to ensure plant survival and reproduction. N availability is one of the factors controlling phase changes. In this review, we integrate and summarize the known effects of N over different developmental stages in plants. Substantial advances have been made in our understanding of signalling and N-responsive gene regulatory networks. We focus on the molecular mechanisms underlying N regulation of developmental transitions and the role of putative new regulators that might link N availability to pathways controlling Arabidopsis growth and development from seed germination through the plant reproductive transition.

Key words: Development, gene regulatory networks, microRNA, nitrate, nitrogen, phase transition, signaling.

Introduction

In contrast to higher animals, embryogenesis in plants is just the basis for further growth. Post-embryonic development continues throughout the life cycle of the plant from stem cells located in shoot and root meristems in order to develop new organs with different physiological and functional capabilities. The ability to constantly create new organs not only allows plants to increase in size and develop into reproductive adults but is also a key mechanism to adapt their morphology and physiology to their environment in order to optimize the acquisition of nutrients and the production of building blocks and energy (Poethig, 2013; Lastdrager et al., 2014).

Higher plants undergo several distinct transitions during their post-embryonic development, including germination, the heterotrophic to autotrophic transition, juvenile vegetative to adult vegetative transition, and vegetative to reproductive transition. The correct timing of these developmental events is critical for survival and reproduction, and is regulated by complex interactions between endogenous and environmental factors (Bernier, 1988; Rougvie, 2005; Amasino, 2010; Huijser and Schmid, 2011; Tolson and Chappell, 2012). Nutrients play important roles in many developmental transitions in both animals and plants. In Caenorhabditis elegans, nutrients regulate larval maturation by controlling the heterochronic pathway while developmental arrest is initiated by nutrient-deficient conditions (Baugh, 2013), and in mammals, nutrient levels affect the onset of puberty (Tolson and Chappell, 2012). Nutrients regulate the timing of metamorphosis in Drosophila through their effect on a nutrient-sensing pathway mediated by TARGET OF RAPAMYCIN (TOR) (Layalle et al., 2008), a protein that is evolutionarily conserved from yeast to plants and humans (Wullschleger et al., 2006; Zoncu et al., 2010; Dobrenel et al., 2011; Russell et al., 2011). In Arabidopsis, glucose controls a TOR-dependent transcriptional network that dynamically represses programmes associated with seed nutrient metabolism for germination and stimulates root meristem activation to control developmental transition and growth (Xiong et al., 2013).
Nitrogen (N) is an essential macronutrient for plants, and a key structural component of macromolecules. N availability is a major limiting factor for plant growth and crop production (Frink et al., 1999). Therefore, N nutrient and metabolite availability, acquisition, metabolism, and use must be tightly coordinated with developmental and growth programmes to ensure plant survival and reproduction. N nutrients such as nitrate, ammonium, and organic N compounds are able to control both local and systemic regulatory networks, in some cases acting as signalling molecules (Vidal and Gutiérrez, 2008; Alvarez et al., 2012; Gutiérrez, 2012). N-responsive gene regulatory networks modulate growth in a tissue- or cell-specific manner to coordinate development and N supply. Although substantial advances in understanding and deciphering N-signalling pathways that control these N-responsive regulatory networks in plants have been made (Krouk et al., 2010a; Tsay et al., 2011; Alvarez et al., 2012; Schachtman, 2012), little is known about how regulation of these networks generates changes in developmental phase transitions that ultimately lead to organ formation and growth. Understanding the regulatory networks that control plant development is the first step towards developing strategies for biotechnological purposes such as improving crop productivity and is of paramount importance for sustainable agriculture in the 21st century.

In this review, we highlight and discuss the role of N nutrients in regulating specific developmental phase transitions of the plant life cycle and the role of potential new molecular candidates for the integration of N signals into plant developmental programmes.

**N regulation of seed germination**

Seeds have the ability to survive long periods of time under unfavourable conditions and remain dormant until environmental conditions are optimal for germination. Successful germination of the seed allows establishment of the seedling and initiation of post-germinative growth. Germination is a complex process in which the seed must shift from a maturation to a germination programme of development in order to prepare for seedling growth. Seed dormancy, a temporary quiescent state that is observed in seeds from many plant species, prevents germination at incorrect times and ensures plant survival by adjusting vegetative development to seasonal changes in the environment (Koornneef et al., 2002; Donohue et al., 2005). Seed dormancy and germination times are highly regulated by both extrinsic and intrinsic cues including plant hormones, such as abscisic acid (ABA) and gibberellins (GAs) (Koornneef et al., 2002; Kucera et al., 2005). Among external cues that have an impact on the timing of seed germination, N availability has been identified as a potent controller of seed dormancy and germination. Seeds of Arabidopsis plants exposed to high-nitrate concentrations or seeds directly exposed to nitrate during imbibition are less dormant than seeds from plants exposed to low nitrate or imbibed in water (Alboresi et al., 2005), indicating a negative correlation between nitrate and seed dormancy. The effect of nitrate on seed dormancy is mediated by reductions in ABA levels brought about by the induction of CYP707A2, a gene involved in ABA catabolism (Fig. 1), by nitrate (Alboresi et al., 2005; Ali-Rachedi et al., 2004; Matakadias et al., 2009). The effect of nitrate over dormancy has been shown to be independent of nitrate reduction and assimilation (Alboresi et al., 2005), indicating that nitrate can act as a signal to control seed dormancy. In this signalling pathway, nitrate would be sensed by the nitrate sensor/transporter NRT1.1 (Alboresi et al., 2005). Expression of this transporter has been shown in dormant seeds and is increased upon seed imbibition and germination (Guo et al., 2001; Finch-Savage et al., 2007). Furthermore, the levels of the CIPK23 kinase that phosphorylates NRT1.1, switching its low nitrate affinity for high nitrate affinity (Ho et al., 2009), are inversely correlated with seed dormancy (Footitt et al., 2013). This suggests that the phosphorylation level of NRT1.1 by CIPK23 might play a role in control of seed dormancy by nitrate. Besides nitrate, nitric oxide (NO), a reactive nitrogenous species that can be synthesized by reduction of nitrite by nitrate reductase (Desikan et al., 2002), has also been shown to promote germination (Batak et al., 2002;
Transcripts for both nitrate reductase genes are expressed in seeds, NIA1 being expressed more highly, particularly during seed dormancy relief by nitrate (Finch-Savage et al., 2007). Incubation of dormant seeds with a NO scavenger increases seed dormancy, while treatment with a NO donor induces seed germination (Bethke et al., 2006). Interestingly, NO has been shown to mediate the relief of seed dormancy caused by other N-related compounds including cyanide, nitrite, and also nitrate (Bethke et al., 2006, 2007). A rapid accumulation of NO, possibly in the endosperm layer, during the first stage of Arabidopsis seed imbibition is required for ABA catabolism and release of seed dormancy (Liu et al., 2009). In addition, NO may control seed dormancy and germination by direct modification of proteins by cysteine S-nitrosylation or tyrosine nitration (Moreau et al., 2010; Arc et al., 2011). Although there are no confirmed targets of NO in seeds that could mediate NO effect over dormancy, many nitrosylated proteins identified in Arabidopsis seeds are involved in metabolic processes (Lindermayr et al., 2005; Romero-Puertas et al., 2008). For example, a β-subunit of the mitochondrial ATP synthase complex is S-nitrosylated in dry Arabidopsis seeds (Arc et al., 2011), indicating that NO might impact on the energy status of the seed. Among proteins subjected to tyrosine nitration identified in Arabidopsis seedlings is ABA3, involved in ABA biosynthesis (Lozano-Juste et al., 2011). Inactivation of ABA synthesis by nitration of ABA3 might lead to dormancy release. Thus, N availability might work as a signal controlling seed dormancy and germination by a pathway involving NO production, interaction with ABA, and perhaps post-translational protein modification.

**N control of heterotrophic to autotrophic transition and early seedling development**

During seed development, C and N resources are stored mainly in the form of lipids such as triacylglycerol (TAG) and proteins in Arabidopsis (Mansfield and Briarty, 1992; Graham, 2008). Upon germination, TAG is hydrolysed by lipases to generate fatty acids. Fatty acids are catabolized via β-oxidation and the glyoxylate cycle pathways to make precursors for biosynthesis of sugars and production of energy for seedling growth (Theodoulou and Eastmond, 2012). At the same time, the storage protein reserves are mobilized mainly in vacuoles generating C skeletons and N that can be used to support seedling growth (Mansfield and Briarty, 1996). In Arabidopsis, this phase of heterotrophic metabolism occurs during the first 48 h after imbibition (Mansfield and Briarty, 1996). Thereafter, a significant reduction of storage reserves occurs, so a rapid switching from heterotrophic to autotrophic metabolism is necessary for the growth and survival of the new seedling. This developmental phase change is marked by differentiation of etioplasts into chloroplasts in cotyledons, allowing seedlings to acquire photosynthetic competence (Shimada et al., 2007). Transition from heterotrophic to autotrophic growth occurs rapidly, between 48 and 96 h after imbibition (Mansfield and Briarty, 1996; Allen et al., 2010). Interestingly, the C:N ratio rather than C or N status of the plant alone influences heterotrophic to autotrophic growth, including seedling growth, storage reserve mobilization, and photosynthetic gene expression (Martin et al., 2002; Sato et al., 2009).

High C over N external availability, an adverse condition for photosynthesis, inhibits seedling growth with participation of a ubiquitin–proteasome system (Sato et al., 2009, 2011). The RING-H2-type ubiquitin ligases ARABIDOPSIS TOXICOS EN LEVADURA 31 (ATL31)/CARBON NITROGEN INSENSITIVE 1 (CNII) and its paralogue ATL6 have been identified as key elements involved in C/N control of autotrophic growth phase transition (Sato et al., 2009). Altered expression of either ATL31/CNII or ATL6 disrupts C/N sensing or response during Arabidopsis early growth, indicating that protein ubiquitination and degradation are key in maintaining plant growth during transition to autotrophy. Proteomics analysis of proteins associated with ATL31/CNII identified several 14-3-3 proteins as putative ATL31 interactors (Sato et al., 2011). 14-3-3 proteins are able to regulate C/N metabolism by directly binding and regulating the activity of enzymes including NITRATE REDUCTASE (NIA) (Bachmann et al., 1996; Lillo et al., 1997), GLUTAMINE SYNTHETASE (GLN) and other enzymes involved in C and N metabolism (Finnemann and Schjoerring, 2000; Comparot et al., 2003). Thus, 14-3-3 proteins might act as scaffolds recruiting C/N-related targets for degradation by ATL31/CNII in the proteasome pathway, coordinating C/N balance to enzyme activity and growth.

In addition to controlling seed germination, ABA is a key regulator of stress-induced developmental arrest post-germination (Lopez-Molina et al., 2001). TAG biosynthesis is tightly regulated by ABA-related transcription factors including ABA-INSENSITIVE3 (ABI3), ABI4, and ABI5 (Zou et al., 1995; Lu et al., 2003; Penfield, 2008; Holman et al., 2009). N limitation is able to control TAG homeostasis during seedling growth by positively regulating TAG content by inducing the TAG biosynthetic genes ACYL-CoA:DIACYLGLYCEROL ACYLTRANSFERASE 1 (DGAT1), DGAT2, and PHOSPHOLIPID:DIACYLGLYCEROL ACYLTRANSFERASE 1 (PDAT1) (Fig. 1) and this effect is potentiated by C (Yang et al., 2011b). The induction of DGAT1, a major determinant of TAG biosynthesis, is controlled by direct binding of ABI4 to the DGAT1 promoter in response to N limitation (Fig. 1) (Yang et al., 2011b). ABI3 and ABI5 also participate in regulating TAG content under low-N conditions by regulating OLEOSIN (OLE1), a key gene involved in TAG accumulation and they may also participate in TAG biosynthesis by positively regulating DGAT1 expression (Yang et al., 2011b). Recent evidence has shown that ABI1 gene expression can be regulated by a microRNA (miRNA)-mediated pathway involving miR172, a key regulatory factor controlling flowering by repression of transcripts of the floral repressors APETALA2, TARGET OF EAT 1 (TOE1), TOE2, TOE3, SCHLAFMUTZE (SMZ), and SCHNARCHZAPFEN (SNZ) (Aukerman and Sakai, 2003; Schmid et al., 2003; Chen, 2004; Wu et al., 2009) ABA is able to repress the expression of miR172b, leading to an increase of its target SNZ, which in turn may bind the promoter of ABI5 (and probably ABI3 and ABI4) to induce its expression and promote heterotrophic to autotrophic developmental
N regulation of miRNAs as an input for vegetative phase change control?

The vegetative phase of growth includes the developmental period between seedling establishment and plant entry into the reproductive stage. This phase is commonly subdivided into the juvenile and adult phases (Huijser and Schmid, 2011; Poethig, 2013). Juvenile and adult phases of the vegetative phase are distinguished by morphological, anatomical, and physiological parameters (Huijser and Schmid, 2011; Poethig, 2013). For example, in Arabidopsis, juvenile leaves are round-shaped with smooth margins, and trichomes are restricted to the adaxial surface. Conversely, adult leaves are ovated and they possess serrated margins and trichomes on both surfaces (Chien and Sussex, 1996; Telfer et al., 1997). Thus, these morphological traits are commonly used as markers of the juvenile to adult transition (Telfer et al., 1997). Moreover, while juvenile vegetative plants are capable of forming true leaves and auxiliary buds, they are unable to respond to cues that can induce flowering such as day length. It is only during the adult vegetative phase when plants acquire the ability to reproduce (Huijser and Schmid, 2011; Poethig, 2013). Vegetative phase change, or the transition from the juvenile to the adult vegetative phase is thus a consequence of endogenous developmental programmes triggered by plant age (Poethig, 1990, 2013). Concerning the molecular mechanisms that control vegetative phase change, studies have revealed that small RNAs play key roles in this process. The major regulator of vegetative phase change is miR156 and the related miR157, two miRNAs conserved throughout the plant kingdom (Axtell and Bowman, 2008). High expression of miR156/miR157 promotes the juvenile phase, while decreased expression of this miRNA accelerates transition into the adult phase in several species including Arabidopsis (Wu and Poethig, 2006; Chuck et al., 2007; Wu et al., 2009; Wang et al., 2011; Shikata et al., 2012). These miRNAs exert their role in phase change via their targets, members of the SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors. SPL genes from the SPL9 clade (SPL9 and SPL15) and the SPL3 clade (SPL3, SPL4, and SPL5) are involved in leaf initiation and vegetative phase change and in flowering time, indicating that miR156/miR157 can have roles in controlling both phase changes (Schwab, 2012).

The role of environmental stimuli in controlling the timing of vegetative phase change is yet to be determined (Poethig, 2010, 2013), but vegetative phase change is expected to be tightly coordinated with the plant internal nutritional status in order to ensure a correct supply of nutrients to the newly formed organs before the plant becomes responsive to stimuli that trigger the entry into the reproductive phase. miR156a and miR156c levels have been shown to be repressed by a factor produced by leaf primordia, as defoliation generates high levels of miR156 (Yang et al., 2011a). Recent work showed that the leaf-derived signal might be glucose or a glucose-derived metabolite, since glucose represses the expression of miR156 genes promoting vegetative phase change (Yang et al., 2013; Yu et al., 2013). These results suggest that products of photosynthesis can act as positive signals for the plant to proceed into the adult phase. Similarly, N limitation can induce the expression of miR156 in Arabidopsis seedlings (Pant et al., 2009; Liang et al., 2012; Fischer et al., 2013). Analysis of the transcriptome of N-limited plants shows that one of the miR156 targets, SPL3, is downregulated (Bi et al., 2007; Krapp et al., 2011), suggesting that a miR156/SPL3 module might act by repressing vegetative phase change under limiting N availability (Fig. 1). Furthermore, miR156 acts as a negative regulator of miR172 by controlling miR172 expression via its targets SPL9 and SPL15 (Wu et al., 2009). Consistently, N starvation represses miR172 in Arabidopsis leaves (Liang et al., 2012; Fischer et al., 2013). However, no changes in transcript levels of miR172 targets are induced by N starvation in Arabidopsis leaves (Bi et al., 2007; Krapp et al., 2011). Besides transcript stability, miR172 is also able to regulate the protein stability of its targets (Aukerman and Sakai, 2003). miR172 regulation by N starvation may therefore affect its targets at the post-translational level to control the timing of vegetative and reproductive phase transition.

N regulation of vegetative to reproductive transition

Plants undergo a major physiological change as they transition from vegetative growth to reproductive development. This transition is a result of responses to various endogenous and exogenous signals that are integrated into flowering regulation (Mouradov et al., 2002; Simpson and Dean, 2002; Song et al., 2013). Five genetically defined pathways have been identified that control flowering. The vernalization pathway, the photoperiod pathway, the gibberellin pathway, the autonomous pathway, and the endogenous pathway (Srikanth and Schmid, 2011). The molecular mechanisms of these pathways have been studied extensively in Arabidopsis and several other flowering plants (Mouradov et al., 2002; Simpson and Dean, 2002; Corbesier and Coupland, 2006). Environmental cues have been found to be key elements affecting flowering in some plants. In particular, abiotic stresses such as salt, drought, heat, cold, and UV stress promote flowering, and this has been interpreted as a strategy that ensures seed production (Chamont et al., 1982; Martinez et al., 2004; Achard et al., 2006). Mineral nutrition is also able to regulate flowering. Plants watered with nutrient-depleted medium flower later than plants watered with full-nutrient solutions (Zhang and Lechowicz, 1994; Pigliucci and Schlitchung, 1995; Van Tienderen et al., 1996; Kolar and Senkova, 2008). However, this effect is ecotype dependent in Arabidopsis, with some ecotypes showing delayed, advanced, or no effect over flowering when exposed to limiting nutrient concentrations (Pigliucci and Schlitchung, 1995).

N has long been known to modify flowering time in plants, with N limitation often inducing early flowering (Klebs, 1913; Dickens and Staden, 1988; Bernier et al., 1993; Loepky and...
Coulman, 2001). In Arabidopsis, plants grown under low-nitrate conditions flower earlier than plants grown in high-nitrate conditions (Castro Marin et al., 2011; Kant et al., 2011; Liu et al., 2013). Expression of genes that regulate flowering is changed accordingly by nitrate availability. The flowering repressor FLOWERING LOCUS C (FLC) is repressed and FLOWERING LOCUS T (FT), LEAFY (LFY), and APETALA1 (API), positive regulators of flowering, are induced in low-nitrate conditions (Fig. 1) (Kant et al., 2011). This also suggests an interaction with the photoperiod and GA pathways for nitrate-regulated flowering. Accordingly, GA levels are increased by low-nitrate treatments and repressed by high-nitrate treatments (Liu et al., 2013). The CONSTANTS (CO) gene, closely related to the photoperiod pathway, is also induced by low nitrate and repressed by high nitrate (Liu et al., 2013), indicating that nitrate can also regulate flowering by this pathway. However, Castro Marin et al. (2011) showed that low-nitrate induced flowering by a pathway that enters downstream of the floral integrators and is independent of photoperiod, GA, and autonomous pathways, indicating that the specific flowering pathways modulated by nitrate are dependent on the specific experimental conditions used for studying this process and the specific concentration of other nutrients present in the medium.

As mentioned above, differential regulation of specific components of the GA and photoperiod flowering signalling pathways by nitrate availability might determine the timing of flowering in plants. Analysis of the transcriptome of Arabidopsis seedlings and shoots in response to transient high-nitrate treatments (3 or 5 mM KNO₃) (Wang et al., 2003, 2004; Scheible et al., 2004) shows that nitrate represses the GID1B GA receptor and induces the GNC and GNL transcription factors, negative regulators of GA signalling (Fig. 1) (Richter et al., 2010). Also, nitrate induces the transcript levels of TEMPRANILLO1 and TEMPRANILLO2 (TEM1 and TEM2), two transcription factors of the photoperiodic pathway that control flowering by repressing FT and the GIBERELLIN-OXIDASE1 and GIBERELLIN-OXIDASE3 (GA3OX1 and GA3OX3) GA biosynthesis genes (Fig. 1) (Osnato et al., 2012). Other members of the photoperiodic pathway, including TOE1 and SMZ, repressors of the floral integrators (Yant et al., 2009), and SPA1-RELATED4 (SPA4), a protein that represses CO expression by affecting its stability (Laubinger et al., 2006) are induced by nitrate, and FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1), an F-box protein that is involved in CO and FT induction (Fig. 1) (Song et al., 2012), is repressed by nitrate. These results are consistent with nitrate availability controlling the GA pathway at different levels (GA biosynthesis, perception, and signalling) and also members of the photoperiod pathway to determine the timing of vegetative to reproductive phase change.

Concluding remarks and future directions

Recent research on the N response of plants has identified important regulators including NRT1.1, as a nitrate sensor (Ho et al., 2009), and NLP7 (Castaiings et al., 2011; Marchive et al., 2013), SPL9 (Krouk et al., 2010b), LBD37/38/39 (Rubin et al., 2009), and TGA1/TGA4 (Alvarez et al., 2014) as transcription factors regulating nitrate-dependent gene expression. However, most of the work identifying these factors has centred on root responses of young seedlings and N-dependent root phenotypes, and little is known of the role of these signalling molecules on developmental phase transitions. As summarized in this review, N availability impacts on the timing of developmental events during the entire life cycle of Arabidopsis, probably by regulating the expression level of genes that are key regulators of phase changes. Although some shared molecular components could participate in controlling these developmental check points, different developmental stages and plant tissues express different sets of transcripts (Schmid et al., 2005), and therefore it is expected that tissue- and cell-development-stage-specific signalling pathways play a role in transducing the nitrate signal into plant developmental programmes. Although progress made in understanding N control of developmental phase transitions has come from the use of molecular genetic techniques on the model species Arabidopsis thaliana, this knowledge could be used to investigate the effect of N in transition phase changes in other plant species. For example, it has been described that miRNAs involved in the control of the floral transition stage are conserved throughout the plant kingdom (Chen et al., 2013; Luo et al., 2013). Designing strategies to control the timing of developmental phase transitions is of pivotal importance for fruit and crop production and also for biotechnological purposes. For example, overexpression of maize miR156 (CORNGRASS1) in switchgrass has been shown to prevent flowering, improve digestibility, and increase starch content (Chuck et al., 2011). Thus, modifying N fertilization of crops can directly impact on shoot biomass content in crops utilized for biofuel production. Specific analysis of the N response of plants during development of different organs and cell types might shed light into N-gene regulatory networks coordinating plant growth and development to N availability.

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