Progress in the regulation of nitrate uptake by plants by Auxin response factors

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Abstract. Nitrogen is indispensable for plant growth, as plants can take up external nitrates to promote the development of the organism. However, improving effective nitrate uptake by plants is a problem that needs to be solved worldwide. Growth hormone plays an essential role in regulating nitrogen utilization in plants, and most of the processes are regulated by ARF family genes. In this paper, the signal transduction mechanism, spatial and temporal expression patterns of ARF family genes and the biological functions of ARF in regulating nitrogen utilization were introduced according to the latest research in China and abroad. Finally, the shortcomings of plant ARF research are analyzed, and the future research direction is prospected.

Keywords: Auxin, ARF, Nitrogen Utilization, Regulatory Mechanism.

1. Introduction

The two primary sources of nitrogen for plants are nitrate and ammonium nitrogen. Nitrate is the primary type of nitrogen in soil when it is aerobic. Nitrate must be reduced and changed into nitrite by nitrate reductase in the cytoplasm before it can bind to amino acids. The nitrite reductase enzyme in the plastids or chloroplasts then reduces the nitrite and turns it into ammonium. Plants are poisoned by excess ammonium and nitrite. As a result, these absorption processes at the cellular level must be carefully regulated in response to nitrogen availability and demand as well as carbon status, which supplies the building blocks for amino acid synthesis [1-6].

Over the past 50 years, the use of nitrogen fertilizers has significantly increased agricultural yields worldwide. However, excessive N inputs have the potential to harm the environment severely and decrease biodiversity. In order to attain a more sustainable agriculture with low nitrogen requirements, it is vital to develop new crop varieties with high yields and increased nitrogen use efficiency (NUE) [3]. There is mounting evidence that some phytohormone signaling pathways, including gibberellins, IAAs, cytokinins, abscisic acid, and monocotyledonolactone, interact with plant nitrogen metabolism to influence plant growth and development [3].

Almost every element of plant growth and development is regulated by the plant hormone. Indole-3-acetic acid (IAA), a highly important natural hormone, is found in plants. This plant hormone is essential for cell division, fruit formation, elongation, and senescence. Roots, leaves, and flowers are

stimulated by IAA. IAA, in particular, causes adventitious root production in monocotyledons and lateral root formation in dicotyledons. Additionally, growth hormones are crucial in encouraging nitrate absorption [7].

Numerous candidate genes potentially subject to regulation by growth factors and implicated in the processes of growth and development have been discerned in Arabidopsis and other plant species. Within this set of genes, it is widely believed that the Auxin response factor (ARF) family members has a significant capacity to govern the expression of genes that respond to auxin [8, 9].

While research into IAA signaling's impact on plant nitrogen uptake is ongoing, there's still much to discover. We need a better understanding of how IAA-related genes respond to fluctuations in nitrogen fertilizer effectiveness. Additionally, it's unclear whether IAA regulates nitrogen metabolism, potentially improving nitrogen fertilizer efficiency in crops. As we identify more transporter proteins and signaling components and understand their functions, we can unlock the strategies that help plants maximize nitrate utilization. This knowledge can inform new approaches for enhancing nitrogen utilization efficiency through genetic modification.

2. IAA in plants

IAA, initially found in plants in the late 19th century, has seen multiple proposed biosynthesis routes over the years. Enzymes catalyzing these reactions and the involved intermediates have been identified to define these pathways [7]. Therefore it is of great interest to study IAA in plants.

Several enzymes have been discovered to play a role in the inactivation of the plant hormone growth hormone, which plays a crucial role in plant development. In the past, it was discovered that the GH3-ILR1-DAO pathway was used to inactivate IAA, the main growth hormone presents de nature. The GH3 IAA-amino synthase first transforms IAA into IAA-amino acid conjugates. The storage forms of IAA are IAA-aspartate (IAA-Asp) and IAA-glutamate (IAA-Glu), which can both be converted back to IAA by the enzyme ILR1/ILL aminohydrolase. The researchers discovered that IAA-Asp and IAA-Glu could be irreversibly oxidized by DAO1 dioxygenase to create 2-hydroxyindole-3-acetic acid-aspartate (oxIAA-Asp) and 2-hydroxyindole-3-acetic acid-glutamic acid (oxIAA-Glu), which could then be hydrolyzed by ILR1 to release inactive oxIAA [7, 10].

The majority of IAA is carried throughout the plant through the phloem, where it accumulates in various tissues and forms specific concentration gradients [11]. IAA is diffused across the cell wall and membrane during polarized transit between cells. IAA's hydrophobic protonated form diffuses through the cell membrane whereas its hydrophilic anionic form does not. As a result, certain IAA influx carriers and efflux transporter proteins actively transport the anionic form.

3. IAA affects nitrate uptake by plants

Nitrogen plays a crucial role in supplying vital nutrients necessary for the growth and development of plants, while hormones serve as significant regulatory agents in the process of plant growth. The relationship between phytohormones and nitrate transporter proteins is mainly manifested in two aspects: hormones are involved in the expression regulation of nitrate transporter proteins; nitrate transporter proteins affect hormone biosynthesis, transportation, and signal transduction. Phytohormones and nitrate transport proteins interact and coordinate with each other and play important roles in the growth and development of plants [3].

The interaction between IAA and nitrate transporter proteins plays a significant role in the process of plant growth and development. The induction of gene expression by IAA facilitates the promotion of nitrate absorption. An illustrative instance is the demonstration of IAA transport activity in oocytes by *AtNPF6.3* (or *NRT1.1*), which may be impeded by nitrate. Furthermore, the gene *NPF6.3* governs the development of lateral roots in reaction to varying external nitrate concentrations by means of nitrate-regulated IAA transport. At the absence of sufficient nitrate, the NPF6.3 protein functioned as a transporter of indole-3-acetic acid (IAA) and hindered the accumulation of IAA at the tip of the lateral root. Consequently, this led to the termination of elongation in the lateral root. The inhibition of IAA

transport by *NPF6.3* in the presence of nitrate resulted in the elongation of lateral roots. This elongation was attributed to the buildup of IAA at the tip of the lateral root, indicating a connection between nutrient and hormone signaling in the context of plant growth [3]. In the context of biomass growth, scholars have used glucose or indole-3-acetic acid (IAA), with particular emphasis on their combined application, to augment plant biomass via the enhancement of root system architecture, facilitation of root nitrogen metabolism, and stimulation of nitrate absorption. To summarize, the addition of glucose to soils that lack sufficient carbon content has the capacity to alter the expression levels of genes associated with the synthesis and transport of growth hormones, resulting in the redistribution of naturally occurring indole-3-acetic acid (IAA) throughout the root system. As a result, this particular mechanism has the potential to improve both the structural composition of the root system and its nitrogen metabolic functions [12].

The study of the growth hormone regulatory network revealed that the nitrogen response module composed of the IAA receptor *AFB3* and *miRNA393* affects plant root conformation by regulating a transcription factor, the NAC4. This protein plays a crucial role as a regulatory component within the nitrate response network, since it governs the efficiency of both external and internal nitrogen assimilation processes in plants. The above results indicate that nitrate transporter proteins are closely related to IAA, the expression of key genes in auxin transport is regulated by nitrate, and some nitrate transporter proteins are involved in growth auxin transport [13].

4. The ARF family in plants

Auxin response factors (ARFs) play a crucial role in the regulation of plant growth and development. The identification of auxin response factors was conducted in the model plant Arabidopsis. A comprehensive set of 23 genes belonging to this particular family were subsequently found in Arabidopsis. The 23 genes are dispersed over the five chromosomes of the organism. The family of ARF gene, which comprises a group of transcription factors, has a modular structure with many conserved structural domains that have remained unchanged over an extensive period of evolutionary time, spanning hundreds of millions of years [8]. ARF proteins consist of a non-conserved structural domain, known as the activation domain (AD) or repression domain (RD), which is surrounded by the N-terminal DNA-binding domain (DBD) and the C-terminal PB1 structural domains. The dimerization structural domain (DD) and the flanking structural domain (FD) are situated at both the N-terminal and C-terminal regions of the B3 domain inside the DBD structural domain. The F-BOX proteins known as transport inhibitor response 1/AUXIN signaling F-BOX proteins (TIR1/AFBs) participate in the formation of the SCFTIR1 ubiquitin-protein ligase complex with other proteins such as ASK1, CUL1, and RBX. When growth hormone levels are low, Aux/IAA proteins have a tendency to aggregate with ARFs, resulting in a significant decrease in the transcriptional efficiency of ARFs. Elevated concentrations of growth hormone facilitate the process of ubiquitination and subsequent degradation of Aux/IAAs via the SCFTIR1/AFB complex and 26S proteasome. This degradation enables the DNA-binding domain (DBD) inside ARFs to interact with AuxRE, therefore enhancing the effectiveness of transcription [14].

5. Rloe of ARFs in plant growth

The regulation of ARF expression at different times in various organs of plants plays an important role in growth and development. Since Arabidopsis thaliana and rice serve as model plants in botany, the ARF family has been studied in depth.

5.1. Expression pattern of ARFs

There are many plant species in the world, and the number of members of the ARF family varies from plant to plant, and their spatiotemporal and temporal expression patterns differ somewhat among species. In Arabidopsis thaliana, *AtARF1* is mainly expressed in flowers, and *AtARF2* is expressed in all tissues [15]. Rice *OsARF18* is mainly expressed in spikelets, and tomato *SlARF3, 5, 6, 9, 13, 15*, and *17* are mainly expressed in flowers and fruits [16]. Environmental conditions such as light, darkness, and some

exogenous hormone treatments also affect the expression of ARF genes. In Arabidopsis thaliana, the transcript levels of ARF family genes were changed in darkness, in which *ARF2, ARF7,* and *ARF19* were up-regulated, while *ARF1* was down-regulated. External growth hormone or ethylene also affected the expression level of *ARF* genes [14].

5.2. Transcriptional activation of ARF transcription factors

The Arabidopsis transcriptional activators, namely AtARF5-8 and AtARF19, exhibit a high abundance of glutamine, serine, and leucine in their amino acid composition. These activators share homology with each other. Extensive investigations involving mutants associated with these activators have demonstrated their significant involvement in various developmental processes, including embryo development, hypocotyl elongation, vascular tissue formation, lateral root formation, flower development, and phototropism. When a moderate concentration of IAA was applied unilaterally to the hypocotyl of Arabidopsis wild plants, the hypocotyl showed bending, whereas when growth hormone was applied unilaterally to the hypocotyl of the Arabidopsis arf7 mutant, the hypocotyl did not bend accordingly, suggesting that AtARF7 plays an important role in the movement toward the light. Arabidopsis AtARF5 is involved in IAA transduction, and AtARF5 promotes transportation of IAA not only to adjacent stem cells but also to the precursor of the quiescent center of the root apical meristem during early embryogenesis [17, 18].

Recent studies have revealed that plants can adopt a low-consumption strategy to enhance nutrient uptake at low nitrate concentration states through the IAA signaling pathway. The present framework encompasses a sequential series of processes including the synthesis, translocation, and signaling of indole-3-acetic acid (IAA), which together stimulate the elongation of root hairs in order to facilitate the acquisition of nitrogen sources by plants. IAA accumulation in the root tip was enhanced in a low-nitrogen environment via the up-regulation of tryptophan aminotransferase in Arabidopsis 1 (TAA1) and YUC8. Subsequently, the indole-3-acetic acid (IAA) was guided into the root hair (RH) differentiation zone via the involvement of growth hormone transporter protein 1 (AUX1) and PIN-FORMED 2 (PIN2) inside the IAA transport apparatus. When the plant enters the reproductive hormone (RH) zone, the IAA triggers the activation of certain transcription factors known as auxin response factors 6 and 8 (ARF6/8). This activation leads to the promotion of the epidermal and indole-3-acetic acid (IAA)-induced transcriptional module called RHD6-LRL3. Consequently, the plant undergoes elongation in the RH zone as a response to low nitrogen levels [19].

5.3. Transcriptional repression of ARF transcription factors

In the context of higher plants, growth and development are reliant on the provision of nutrients by photosynthetically active organs. The allocation of carbohydrates between source and sink tissues is a crucial factor in the regulation of plant growth and development. The conclusions of the study revealed that the rice mutant *dao* exhibited spikelet closure and fruiting problems due to the transcriptional activation of *OsARF18* and transcriptional suppression of *OsARF2*. The primary reason for this phenomenon may be attributed to the robust activation of rice *OsARF18* in the presence of elevated levels of indole-3-acetic acid (IAA), which subsequently leads to the suppression of the expression of *OsARF2* and *OsSUT1*. This repression is achieved by direct binding of *OsARF18* to the AuxRE and SuRE motifs located within the promoter regions of *OsARF2* and *OsSUT1*, respectively. Following this, the process of carbohydrate partitioning was also hindered, resulting in the failure of spikelets to open [20].

The presence of the *DNR1^{indica}* variant allele is associated with a reduction in the levels of DNR1 transcripts and proteins, leading to an accumulation of growth hormones. Consequently, the aforementioned process results in the activation of genes associated with nitrogen metabolism and the reduction of tiller growth, facilitated by *arf*. Therefore, rice that has the *DNR1^{indica}* mutation exhibits a substantial yield and a reduced need on nitrogen [21].

The *rst1* mutant, characterized by enhanced salt tolerance and grain output, represents a significant advancement in the field of rice cultivation. The findings indicate that the *rst1* mutant exerts a direct

influence on the upregulation of the asparagine synthase 1 gene. This regulatory mechanism leads to the production of asparagine and prevents excessive accumulation of ammonium. Consequently, plant growth and yield are enhanced under normal conditions, while yield loss is mitigated under salt stress. Additionally, the mutant promotes nitrogen utilization efficiency [22].

The process of fruit development is a crucial reproductive mechanism in higher plants. It typically begins with a significant phase of cell division, which is subsequently followed by cell expansion. This cellular growth is essential for creating a favorable environment that facilitates the maturation of the developing seeds inside the fruit. The primary hormone responsible for stimulating fruit development is IAA. The *SlARF7/SlIAA9* complex in tomato serves as a repressor complex for IAA signaling. Additionally, both *SlARF7/SlIAA9* and *SlDELLA* act as antagonists to the expression of regulated genes, such as GA biosynthesis genes (*GA20ox1* and *GA3ox1*) and growth hormone inactivation genes (*GH3.2*). Consequently, these actions inhibit the early development of the fruit-sitting box [9].

6. Target genes of *ARF* and their regulatory mechanisms

ARF transcription factors regulate plant growth and development by activating or repressing their target genes in response to growth factors or other environmental conditions. The target genes of ARF are mainly genes that are rapidly induced by growth factors, including the *Aux/IAA* gene, *SAUR* gene, and *GH3* gene. The DBDs of ARF bind to the TGTCTC/GAGACA sequence of the same AuxRE element to activate or repress the expression of the target genes. As an example, RST1 has been shown to have an influence on the transcriptional activity of *OsARF18* by directly interacting with the promoter region of *OsAS1*, therefore leading to the repression of its production. Additionally, *OsARF18* has been found to bind to the AuxRE element of *OsARF2*, resulting in the repression of its expression. The transcription factor *AtARF7,19* exerts direct activation on certain *LBD/ASL* genes, hence regulating the developmental processes associated with lateral root formation in Arabidopsis [8].

7. Conclusions

The transcription factor ARF is closely linked to the auxin response factor (IAA) in several aspects of plant growth and development. Differences in growth hormone content lead to differences in nitrogen utilization efficiency and yield of plants. Therefore, regulating growth hormone content through the ARF family is a promising strategy to reduce the environmental degradation of agricultural nitrogen inputs in rice while sustainably increasing grain yield.

Acknowledgments

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