Review

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Insights into molecular links and transcription networks integrating drought stress and nitrogen signaling

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Summary

Drought and the availability of nitrate, the predominant source of nitrogen (N) in agriculture, are major factors limiting plant growth and crop productivity. The dissection of the transcriptional networks' components integrating droght stress and nitrate responses provides valuable insights into how plants effectively balance stress response with growth programs. Recent evidence in *Arabidopsis thaliana* indicates that transcription factors (TFs) involved in abscisic acid (ABA) signaling affect N metabolism and nitrate responses, and reciprocally, components of nitrate signaling might affect ABA and drought gene responses. Advances in understanding regulatory circuits of nitrate and drought crosstalk in plant tissues empower targeted genetic modifications to enhance plant development and stress resistance, critical traits for optimizing crop yield and promoting sustainable agriculture.

I. Introduction

Drought stress is closely associated with water deficit, a condition that arises when a plant's transpiration rate exceeds its water absorption capacity due to insufficient water, increased salinity, or osmotic pressure (Bray, 1997; Vishwakarma *et al.*, 2017). Dehydration triggers osmotic and hormone-driven signals, including an increase in abscisic acid ABA, a hormone that plays a significant role in the plant's response to drought (Zhang *et al.*, 2006; Vishwakarma *et al.*, 2017).

In general, drought stress negatively impacts plant growth (Fig. 1a). The prevailing tension between growth and drought resistance is often attributed to energy and resource constraints: Stressed plants must divert energy and resources away from growth toward stress response mechanisms. During the initial stages of the stress response, the stress signaling network actively inhibits cellular anabolic processes and overall plant growth, even if the cellular energy status remains stable (Zhang *et al.*, 2006; Vishwakarma *et al.*, 2017).

One critical aspect of plant growth prominently suppressed by drought is nitrogen (N) metabolism and the signaling of nitrate, the predominant source of N in agricultural systems (Araus *et al.*, 2020; Plett *et al.*, 2020). In contrast to drought, nitrate is a positive nutrient signal for plant growth and development (Fig. 1a). Nitrate

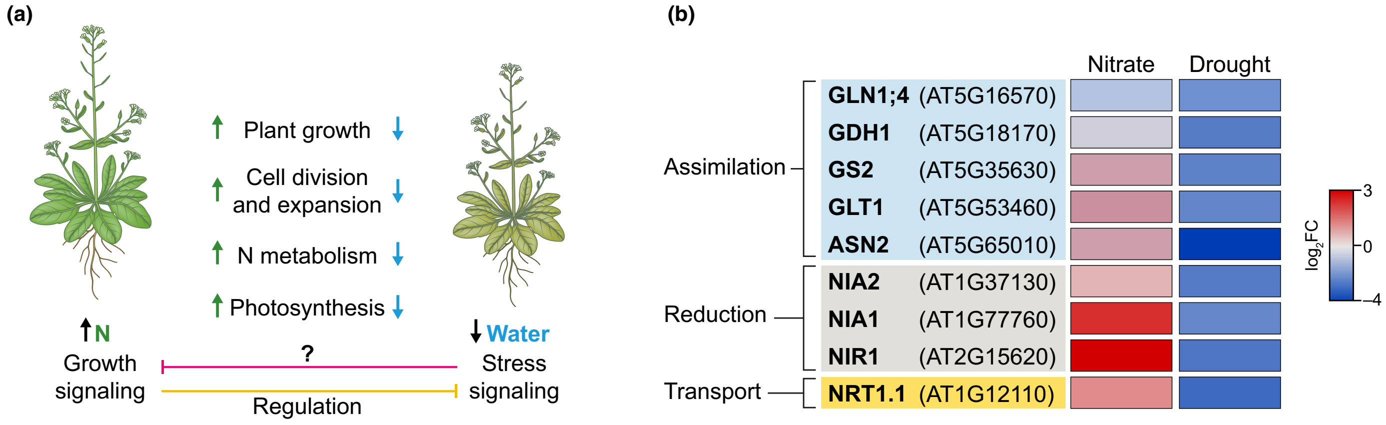

Fig. 1 Drought and N have opposite effects on major physiological plant traits. (a) Through stress sensing and signaling, drought stress actively inhibits traits related to growth (blue arrows) and negatively impacts growth signaling (purple path). When N is abundant, and stress is absent, N-related growth signaling activates growth traits, repressing stress signaling (yellow path) and promoting growth. The mechanisms underlying this reciprocal regulation are poorly understood. (b) Genes involved in nitrate uptake, reduction, and assimilation are induced by nitrate and repressed by drought, according to (Canales *et al*., 2014; Sharma *et al*., 2018), respectively. This contrasting influence on gene expression might correspond to the growth-enhancing role of nitrate and the growth-restricting effect of drought.

supply rapidly affects the expression of a myriad of genes that will produce changes in root architecture, shoot development, and increase plant biomass (Gaudinier *et al*., 2018; Y. Y. Wang *et al*., 2018; Vidal *et al*., 2020; Li *et al*., 2021). Drought stress can alter the expression of genes involved in N metabolism (Araus *et al*., 2020; Fig. 1a). As depicted in Fig. 1(b), genes associated with nitrate transport/sensing, reduction, and assimilation are upregulated by nitrate, while they are downregulated in response to drought treatments. These genes are responsible for converting N from various sources, such as ammonia and nitrate, into forms that can be used by the plant. When their expression level is reduced, the plant's ability to assimilate N is severely compromised (Fig. 1a,b).

Developing research strategies for improving drought tolerance in plants under adequate nitrate levels is crucial yet intricate. We recommend referring to the following reviews that have focused on the physiological aspects of the nitrate and drought interaction and proposed research strategies for improving drought tolerance under changing nitrate availability (Ullah *et al*., 2019; Araus *et al*., 2020; Plett *et al*., 2020).

This review highlights the genetic aspects of nitrate and drought interaction, along with the transcriptional networks involved in mediating the interactive effects between these signals using the abundance of genomic data in *Arabidopsis thaliana*. Unraveling the regulatory mechanism controlling drought and nitrate responses is fundamental to devising innovative strategies that can simultaneously enhance plant drought tolerance while maintaining vigorous growth (Ding *et al*., 2018; Plett *et al*., 2020).

II. Molecular links between N, ABA, and drought signaling

One of the key players influencing growth and stress response is the ABA phytohormone. This hormone not only mediates essential developmental processes such as seed maturation and dormancy but also plays a critical role in stress responses, such as controlling stomatal aperture during drought, promoting leaf senescence, and inhibiting growth (Yoshida *et al*., 2014). For instance, plants regulate water usage during drought stress through ABA-mediated processes, leading to stomatal closure and traits negatively impacting growth, including decreased photosynthetic rate, reduced carbon consumption, and stunted growth (Lim *et al*., 2015).

The evolutionarily conserved target-of-rapamycin (TOR) kinase coordinates cellular and organismal growth in eukaryotes. In plants, TOR integrates stress and N nutritional signals. TOR regulates plant stress responses by phosphorylating PYL ABA receptors, preventing stress activation in the leaf under non-stressed conditions. Under stress, ABA-activated SnRK2s phosphorylate Raptor, inhibiting TOR and promoting stress responses (P. Wang *et al*., 2018; Fig. 2). Conversely, organic and inorganic N sources activate the small GTPase Rho-related protein from plants (ROP2), which in turn activates TOR (Liu *et al*., 2021; Fig. 2). Thus, the ROP2-TOR axis may sense and transduce N signals to stimulate leaf growth. Given that TOR is inhibited by stress and activated by N, this could be a mechanism that coordinates leaf growth to optimize plant resources.

In roots, ABA directly inhibits nitrate sensing and transport. Nitrate is perceived and transported inside the root cell by the nitrate transceptor (a transporter and sensor), NRT1.1 (also known as CHL1 or NPF6.3; Ho *et al*., 2009). Notably, ABA signaling modulates NRT1.1 activity. The phosphatase ABI2, which ABA inhibits, indirectly oversees NRT1.1/NPF6.3 activity. Within this regulatory cascade, ABI2 dephosphorylates CBL1–CIPK23, the complex responsible for NRT1.1/NPF6.3′ phosphorylation and subsequent inhibition (Léran *et al*., 2015; Fig. 2). Stress-induced ABA production inactivates ABI2, triggering NRT1.1/NPF6.3 phosphorylation and thus reducing nitrate absorption. This is corroborated by the similar phenotype of *abi2* and *nrt1.1* mutants, both failing to stimulate lateral root growth in the presence of nitrate. The binding of ABA to its receptors, resulting in ABI2

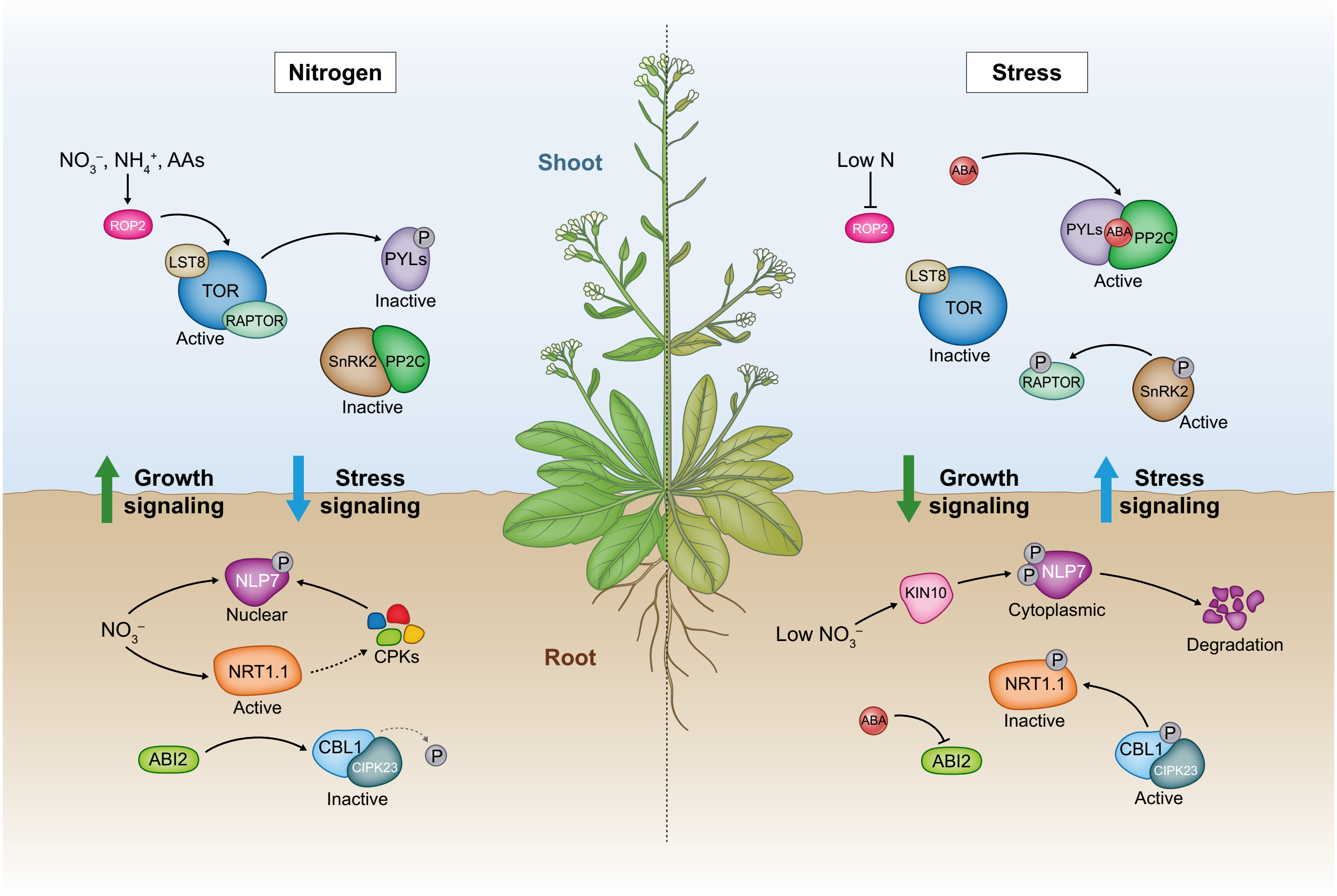

Fig. 2 General schematic illustrating the key proteins mediating balance between growth and stress responses. The diagram depicts proteins reacting to nitrogen (N) stimuli, primarily nitrate (NO_3^-), or stress mainly through abscisic acid (ABA)-dependent mechanisms, underscoring a balance between growth and stress responses in the shoot and root of *Arabidopsis thaliana*. In optimal N conditions in leaves (upper left panel), ROP2 protein is activated in response to various N sources, which in turn activates the TOR complex. In its active configuration, this complex phosphorylates PYL ABA receptors (PYLs), preventing the activation of stress-related signaling under optimal growth conditions (e.g., SnRK2 activation). Under stress conditions (upper right panel), SnRK2 is phosphorylated and consequently activated via ABA-dependent mechanisms, involving the association of PYLs with PP2C phosphatase effectors. Once activated, SnRK2 phosphorylates RAPTOR, a key component of the TOR complex, leading to its dissociation and inactivation. Additionally, ROP2 is inhibited under nutritional deficient conditions (e.g., low N). These processes collectively amplify stress responses and inhibit growth mechanisms in leaves. In roots (lower left panel), nitrate is perceived and transported by NRT1.1, which triggers the activation of a series of calcium-sensor protein kinases (CPKs). These kinases phosphorylate NLP7, a master transcription factor (TF) and nitrate sensor, ensuring its retention in the nucleus. Simultaneously, the phosphatase ABI2 keeps the CBL1–CIPK23 complex in its inactive state. As a result, pathways stimulating root growth are promoted. Conversely, under stress conditions (lower right panel), ABA inhibits the phosphatase ABI2, allowing the CBL1–CIPK23 complex to remain phosphorylated and active. As a result, NRT1.1 is inactivated through phosphorylation by this complex. Furthermore, under nitrate-depletion or stress conditions, KIN10 phosphorylates NLP7 at residues that promote its cytoplasmic retention and degradation, thus inhibiting nitrate-mediated gene expression and plant growth.

inactivation, could be a mechanism to decrease nitrate uptake and lateral root expansion under stress.

Once it is transported and sensed by NRT1.1 in roots, nitrate activates group III calcium-sensor protein kinases (CPKs), which phosphorylates the master TF NIN-LIKE PROTEIN (NLP7) to retain it in the nucleus (Marchive *et al.*, 2013; Liu *et al.*, 2017). Besides NRT1.1 acting as a nitrate sensor in the cell membrane, NLP7 itself can bind nitrate, acting as an intracellular nitrate sensor (Liu *et al.*, 2022). Nitrate directly interacts with NLP7 and induces a conformational change in NLP7, de-repressing it to activate transcription (Liu *et al.*, 2022). Once NLP7 enters the nuclei of root cells, it triggers a rapid transcriptional cascade, inducing primary and secondary gene expression responses (Alvarez *et al.*, 2020).

The energy and stress sensor, SnRK1, regulates nitrate signaling via NLP7, especially in conditions of nitrate and carbon deficiency. KIN10, which is the α-catalytic subunit of SnRK1, phosphorylates NLP7. This action relocates NLP7 to the cytoplasm, leading to its degradation (Wang *et al.*, 2022; Fig. 2). This mechanism contributes to integrating changes in carbon and nitrate availability, reducing nitrate signaling during a carbon shortage under stressful conditions.

Interestingly, both nitrate sensors, NRT1.1 and NLP7, are highly expressed in stomata, a primary site of drought and ABA signaling integration. Indeed, *nrt1.1* and *nlp7* knockout mutants exhibit drought tolerance but are smaller in size than wild-type (WT) plants (Guo *et al.*, 2003; Castaings *et al.*, 2009), reinforcing the notion that achieving drought tolerance may entail a trade-off with N-mediated growth. NRT1.1 is instrumental in regulating stomatal opening and closure, thereby linking nitrate sensing with the drought response. Guo *et al.* (2003) demonstrated this dual role of NRT1.1 in nitrate sensing and drought signaling, showing that NRT1.1 mediated nitrate-induced stomatal closure in Arabidopsis plants exposed to drought stress (Guo *et al.*, 2003). Additionally, NLP7 genome-wide targets are enriched in drought-responsive genes (Araus *et al.*, 2020), suggesting that it not only controls N signaling but also modulates plant responses to drought, likely within stomata.

III. Gene regulatory networks integrating drought stress and N signaling

Over the past 20 yr, transcriptomics has been extensively employed to investigate the complex mechanisms by which plants perceive changes in nitrate, ABA, or drought stress at the molecular level. Comprehensive genomic analyses have revealed that thousands of genes display differential expression when faced with these treatments (as reviewed in Sharma *et al.*, 2018; Araus *et al.*, 2020; Vidal *et al.*, 2020). Prior meta-analyses have provided lists of genes consistently responsive to nitrate and drought, allowing the observation of core biological processes regulated by each treatment (Canales *et al.*, 2014; Sharma *et al.*, 2018).

A recent meta-analysis proposed that Arabidopsis employs the same genes to respond to both N and drought signals. Specifically, 51% of the genes differentially expressed due to N changes also respond to drought. This overlap is larger than expected by chance and remains consistent regardless of the N source. The study also disclosed that genes responding promptly to nitrate changes also respond to drought, and genes responding to drought intersect with nitrate-responsive genes, particularly those enriched in ABA-responsive genes (Araus *et al.*, 2020). These findings suggest the presence of convergent regulatory circuits controlling nitrate, ABA, and drought responses. In other words, transcriptional networks managing nitrate responses also influence ABA- and drought-responsive genes, and transcription factors guiding drought responses also impact nitrate responses.

To explore this hypothesis, we conducted a meta-analysis, taking advantage of available sets in Arabidopsis detailing nitrate-responsive, ABA, or drought-responsive genes and platforms that provide access to extensive, validated TF-target gene interactions.

The recently developed platform, ConnecTF, integrates genome-wide datasets based on TF-target binding (Chromatin Immunoprecipitation and sequencing (ChIP-seq) or DNA Affinity Purification and Sequencing (DAP-seq); O'Malley *et al.*, 2016) and TF-target regulation in Arabidopsis. ConnecTF facilitates the generation of biological insights, such as integrating lists of differentially expressed genes across various treatments and identifying TFs most likely to control these genes based on overlapping significance (Brooks *et al.*, 2021). ConnecTF houses ChIP-seq data that capture *in vivo* TF binding to gene promoters for many TFs in the ABA signaling pathway (Song *et al.*, 2016). It also provides TF-regulation data for 33 TFs in the nitrate-signaling pathway, as recorded by the Transient Assay Reporting Genome-wide Effects of Transcription (TARGET; Brooks *et al.*, 2019), a cell-based assay that identifies direct TF-regulation genome-wide (Bargmann *et al.*, 2013).

In this meta-analysis, we separate drought-responsive genes by ABA-dependent and ABA-independent. We overlapped drought-responsive (Sharma *et al.*, 2018) with ABA-responsive (Song *et al.*, 2016) genes, and such genes were considered ABA-dependent. Conversely, the list of genes in the symmetric difference between the two sets was considered ABA-independent.

To ascertain whether ABA-dependent and ABA-independent gene expression in response to drought is influenced by TFs engaged in nitrate signaling, we compared the list of target genes captured by TARGET for 33 nitrate TFs (Brooks *et al.*, 2019) with the list of genes regulated by either treatment. The results of this meta-analysis reveal that the nitrate-responses cluster with ABA-dependent drought responses; ABA-independent are a separate cluster. The targets of 14 out of 33 TFs (from CDF1 to NAP) are significantly enriched (*P*-value < 1E-40, Fisher's exact test) in ABA-dependent responses and nitrate-responsive genes (Fig. 3a).

Transcription factors involved in nitrate signaling, including known TFs that regulate nitrate-related phenotypes such as TGA1 (Alvarez *et al.*, 2014), HHO2 (Maeda *et al.*, 2018), NAP (Alvarez *et al.*, 2019), and LBD37 (Rubin *et al.*, 2009), also govern drought ABA-dependent responses. Strikingly, none of the TFs involved in nitrate signaling show high enrichment in ABA-independent genes. These results suggest that influential TFs for nitrate response modulate ABA-dependent rather than ABA-independent drought responses (Fig. 3b).

Conversely, we assessed whether gene expression in response to nitrate is influenced by TFs involved in ABA-dependent and ABA-independent signaling. To achieve this, we compared the list of TF-bound genes via ChIP-seq for 21 TFs regulated by ABA (Song *et al.*, 2016) with genes consistently regulated by nitrate (Canales *et al.*, 2014). Genes bound by most TFs show significant enrichment in ABA-dependent and ABA-independent drought-responsive genes (*P*-value < 1E-50, Fisher's exact test; Fig. 4a). Remarkably, a significant overlap was found for 18 out of 21 ABA-TF-bound genes with nitrate-regulated genes, suggesting a role for ABA-dependent and ABA-independent signaling TFs in regulating nitrate-responsive gene expression (Fig. 4b). Previous studies have found regulatory connections between ABA-dependent pathways and nitrate signaling. For example, Nero *et al.* (2009) found enrichment of the ABRE *cis*-motif, recognized by ABA activated TFs, in the promoter of nitrate-responsive genes. A recent study demonstrated convergent regulatory connections between ABA and nitrate signaling through the ABA Response Element Binding/Abscisic Acid Responsive Element Binding Factor (ABF)2 and ABF3 (Contreras-López *et al.*, 2022).

Building upon these findings, a critical role for ABF2 and ABF3 in orchestrating nitrate responses has been further unveiled, particularly within the root endodermis – a pivotal site for nitrate signaling (Contreras-López *et al.*, 2022). A comprehensive analysis of TF-target interactions using Yeast One-Hybrid (Y1H),

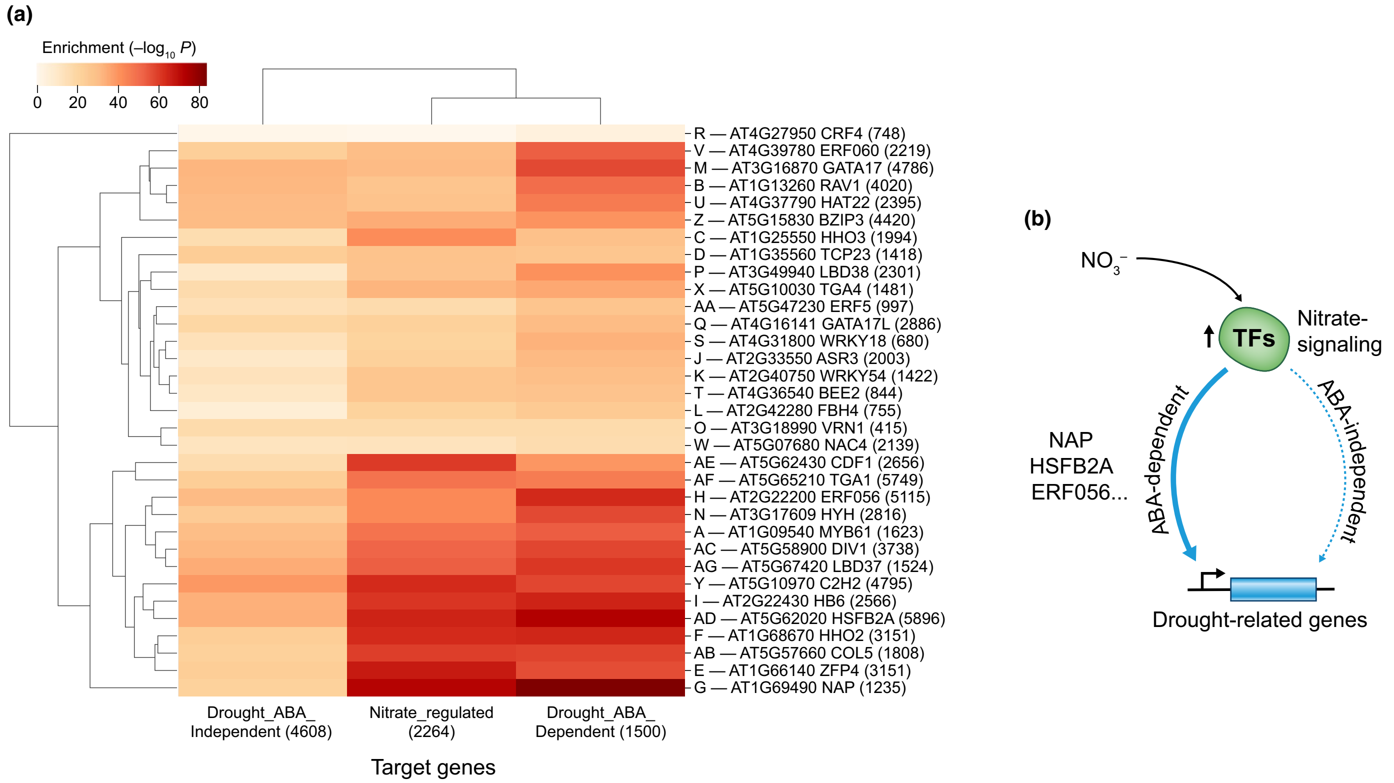

Fig. 3 Genes regulated by transcription factors (TFs) of the nitrate-response are highly enriched in abscisic acid (ABA)-dependent drought-responsive genes. (a) Three published transcriptomic datasets were collected in Arabidopsis that represent consistently nitrate-regulated genes (Canales *et al*., 2014), ABA-regulated genes (Song *et al*., 2016), and drought-responsive genes (Sharma *et al*., 2018). ABA-dependent genes were obtained by overlapping drought-responsive with ABA-responsive genes, and ABA-independent genes were obtained from the symmetric difference between the two sets. These lists were intersected with genes regulated by 33 nitrate-responsive TFs using the TARGET genome-wide assay in isolated cells (Brooks *et al*., 2019). This analysis was done using the *Target List Enrichment* tool in the ConnecTF platform (Brooks *et al*., 2021). ID and symbol of each TF and the number of TF-bound genes (parentheses) are indicated. (b) A summarized schematic illustrates that TFs induced by nitrate tend to preferentially regulate drought in an ABA-dependent manner (blue arrow). This occurs to a much lesser extent with genes related to drought that are ABA-independent (blue dashed arrow).

ChIP-seq, and TARGET assays revealed that ABF2 and ABF3 regulate a significant number of nitrate-responsive genes within the endodermis, including known nitrate-TFs such as TGA1, LBD38, and HRS1. This evidence emphasizes the role of ABA signaling via ABF2 and ABF3 as early, cell type-specific regulators in the nitrate response (Contreras-López *et al.*, 2022). ABF2 and ABF3 also have reported functions in drought tolerance; thus, they would serve as key coordinators integrating nitrate, ABA, and drought stress signals.

How do N, ABA, and drought signals intersect at the regulatory level in crops? The scope of genomic data for these crops is less extensive than that for Arabidopsis, limiting our ability to conduct a similar meta-analysis. Nonetheless, recent research has revealed regulatory links between N, ABA, and drought in rice. Specifically, the rice ABF1 (OsABF1) TF plays a role in governing the expression of genes influenced by both drought and N (Shanks *et al.*, 2022). OsABF1 functions within a network module that bridges gene expression to phenotypic responses under conditions of N and drought (Shanks *et al.*, 2022).

Collectively, existing research, together with new analyses conducted in this review, suggest a persuasive conclusion: nitrate-signaling genes exert influence on ABA signaling and drought-responsive gene expression mechanisms. The recent comprehensive genomic analyses underline the importance of exploring the complex signaling interaction between nitrate and drought signals.

IV. Conclusion

Both nitrate and drought are potent signals that modulate gene expression. While the signaling cascades and TFs governing each response have been deeply explored, our understanding of how these elements interconnect is still emerging. Here, we delve into the established molecular connections between nitrate and drought, highlighting potential transcriptional networks that link these signals.

The known molecular links have been mainly described at the level of protein phosphorylation and modulation of protein function. In leaves, stress suppresses TOR via ABA signaling, while N activates TOR through ROP2 stimulation. In roots, the ABA signaling elements, ABI2 and KIN10, directly influence and alter the phosphorylation status of the nitrate sensors NRT1.1 and NLP7, respectively. These observations imply a potential functional connection between the TOR and ABA signaling pathways, linking stress-induced growth regulation with nitrate uptake and

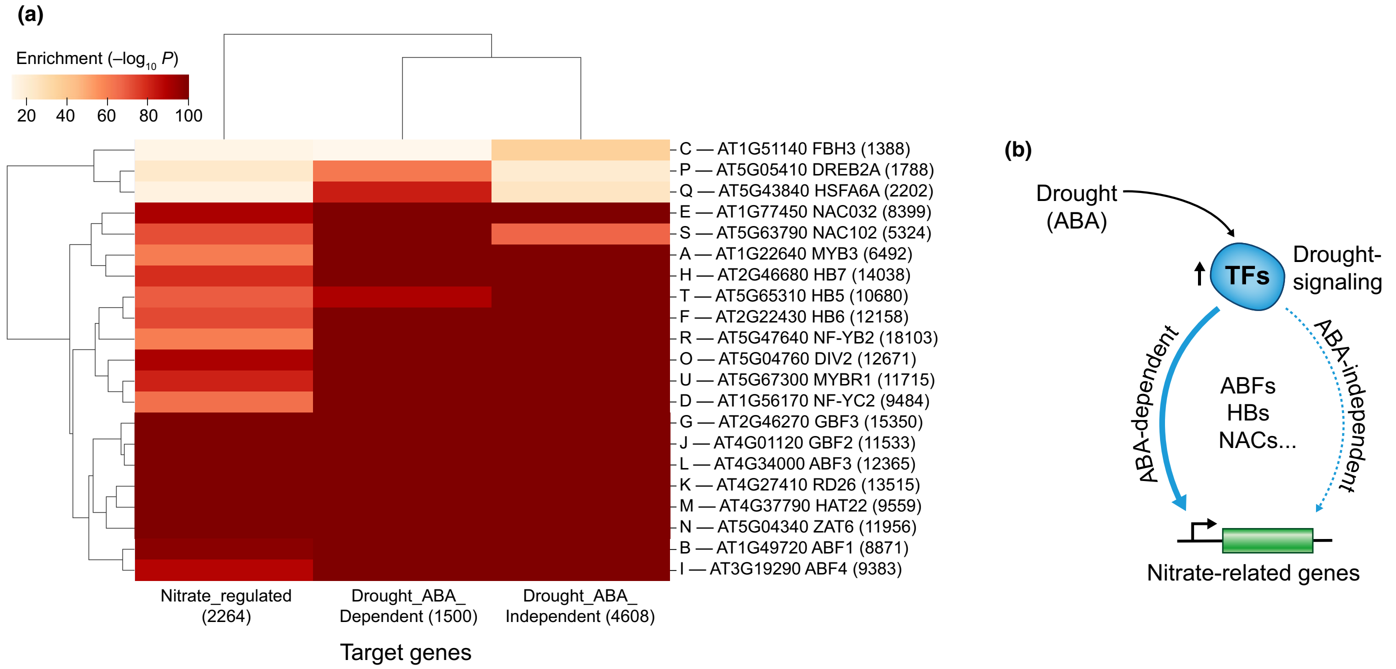

Fig. 4 Genes bound by transcription factors (TFs) involved in abscisic acid (ABA) signaling are highly enriched in N-responsive genes. (a) Genes regulated by N, ABA, and drought treatments in Arabidopsis were collected from Canales *et al*. (2014), Song *et al*. (2016), and Sharma *et al*. (2018), respectively. The intersection of genes regulated by each treatment and genes bound by 21 ABA-responsive TFs captured by ChIP-seq (Song *et al*., 2016), was performed using the *Target List Enrichment* tool in the ConnecTF platform (Brooks *et al*., 2021). The results of the overlaps and their significance are presented as a heatmap. ID and symbol of each TF and the number of TF-bound genes (parentheses) are indicated. Eighteen out of 21 ABA-TFs (FBH3, DREB2A, and HSFA6A are not enriched) are highly enriched in N-regulated genes. (b) The schematic shows that ABA-dependent and ABA-independent pathways activated by drought equally contribute to modulating gene responses to nitrate.

signaling. How leaves respond to root-borne signals originating from nitrate and drought sensing remains unknown.

Our meta-analysis suggests that transcriptional regulation may be a nexus between nitrate and drought signaling. Indeed, we postulate that gene responses to both nitrate and drought involve overlapping regulatory circuits. Our analysis revealed that TFs influencing both ABA-dependent and ABA-independent drought responses also affect the expression of nitrate-responsive genes. Conversely, TFs central to nitrate responses exclusively influence ABA-dependent drought responses. The outcome of altering TFs from one pathway and assessing its influence on the other genome-wide within a unified experimental framework has yet to be established. Undertaking this approach could offer deeper insights into how plants orchestrate transcriptional processes to tailor organ responses to both drought and changes in nitrate levels.

We speculate that as more transcriptomic and TF-target interaction data in different crops grows, network analyses will be performed to uncover transcriptional connections between nitrate and drought responses. Exploring such networks will help design strategies to alleviate the trade-off between growth and stress tolerance and develop crop varieties that thrive in drought conditions and utilize N efficiently.

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Competing interests

None declared.

Author contributions

AC and JMA wrote the manuscript. JMA performed the data analysis. AC designed and made the figures. All authors contributed to the editing and revising of the final version of the manuscript.

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