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Title

Nitrate uptake and its regulation in relation to improving nitrogen use efficiency in cereals

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Abstract

On average less than half of the applied N is captured by crops, thus there is scope and need to improve N uptake in cereals. With nitrate (NO_3^-) being the main form of N available to cereal crops there has been a significant global research effort to understand plant NO_3^- uptake. Despite this, our knowledge of the NO_3^- uptake system is not sufficient to easily target ways to improve NO_3^- uptake. Based on this there is an identified need to better understand the NO_3^- uptake system and the signalling molecules that modulate it. With strong transcriptional control governing the NO_3^- uptake system, we also need new leads for modulating transcription of NO_3^- transporter genes.

Keywords

Nitrate transporters, nitrate signalling, regulation, nitrogen use efficiency, regulation

1. Introduction

Approximately 80 million tonnes of N fertiliser is applied to cereals globally to maximise yields [1]. Unfortunately, the applied nitrogen fertiliser is not used efficiently, with, on average, less than 40% of the applied N being taken up by cereals [2, 3]. This inefficient usage comes at considerable environmental cost and considerable effort is now being directed at improve nitrogen use efficiency (NUE) [4].

The major sources of N in agricultural soils are nitrate (NO_3^-) and ammonium (NH_4^+) [5]. Proportionally NH_4^+ is on average 10% of the soil NO_3^- concentration, making NO_3^- the predominant form of N available to cereal crops [6]. Due to its negative charge and solubility NO_3^- is highly mobile, and in cropping soils can vary by four orders of magnitude from micromolar to millimolar [7]. As sessile organisms, plants therefore need to be able to rapidly adapt to these variable soil NO_3^- concentrations to optimize N capture. In order to enhance the ability of plants to capture the applied nitrogen fertiliser, it is important to understand the processes by which plants acquire NO_3^- and how this process is regulated. This review details current knowledge of these processes, and given their importance in terms of nitrogen application, will where possible relate model plant data to cereals.

2. Nitrate uptake

To cope with such variable soil NO_3^- concentrations plants have two NO_3^- uptake systems: a high affinity transport system (HATS) which is active when NO_3^- in the soil is low (< 250 µM); and a low affinity transport system (LATS) which predominates at high soil NO_3^- concentration (> 250 µM) [8-10]. This has been the accepted paradigm for many years, however recent studies have shown the HATS respond to plant N demand and contribute the majority of total uptake capacity at high NO_3^- concentrations (> 2.5 mM) raising questions regarding the roles and activity of each uptake system [11, 12]. In Arabidopsis these LATS and HATS uptake systems have been linked to the NO_3^- transporter (NRT) families NRT1/NPF and NRT2, respectively, with NRT1.1/NRT1.2 (NPF6.3/NPF4.6) and NRT2.1/NRT2.2/NRT2.4/NRT2.5 primarily mediating NO_3^- uptake [13-

19]. However due to the dichotomy in the *NRT* gene families of dicots and grass species, and the subsequent lack of directly orthologous gene pairs, the function of these genes cannot simply be extrapolated into cereals based on sequence homology [20].

The most extensively studied *NRT* gene is *NRT1.1* (*CHL1/NPF6.3*) which in Arabidopsis is predominantly expressed in the epidermis of young root tips [19]. This gene is NO_3^- inducible and encodes a dual affinity transporter with both HATS and LATS activity [21-24], and also acts as a transceptor with the ability to sense external NO_3^- and activate NO_3^--signalling pathways [25, 26]. The AtNRT1.1 crystal structure reveals that it dimerises in the plasma membrane and operates as a phosphorylation-controlled dimerization switch [23, 24]. Some cereal species have been shown to possess additional *AtNRT1.1* orthologues although their functional roles are yet to be defined [27, 28]. Four co-orthologues have been identified in maize of which three showed different expression patterns and responses to NO_3^- concentration over the lifecycle of maize [11]. Similarly in wheat, four co-orthologous genes were recently identified and shown to have different tissue specificity and transcriptional responses to N supply [28], further confirming that the functional roles need to be separately defined for cereals. In rice a number of co-orthologues have been identified with over expression of one orthologue leading to improved NUE [29, 30]

In contrast to *NRT1.1*, *NRT1.2 (NPF4.6)* expression in Arabidopsis is primarily located in root hairs and the epidermis of both young root tips and mature root regions and constitutively expressed [31]. In cereals a single orthologous *NRT1.2* gene has been identified for each of the sequenced cereal species meaning function may be more evolutionarily conserved [27]. In maize Garnett et al. [11] showed little difference in transcript levels of *ZmNRT1.2* between plants grown at high and low NO_3^- concentration until late reproductive growth where expression profiles differed between treatments. More recently however, a wheat orthologue has been shown to be dramatically induced under N starvation [32], again highlighting the need for complete functional characterisation to confirm this genes contribution to NO_3^- uptake in cereals.

In Arabidopsis *NRT2.1* and *NRT2.2* share 90.4 % sequence identity and are located in tandem on chromosome 1 suggesting they are a product of a gene duplication event [33]. Despite their similarity, AtNRT2.1 has been demonstrated as the main component of the HATS under many conditions with AtNRT2.2 providing only a minor contribution [17, 34]. However, when *AtNRT2.1* is knocked-out *AtNRT2.2* transcript levels have been shown to increase and provide a greater contribution to HATS, partially compensating for the AtNRT2.1 loss [17]. Although the cereal orthologues are yet to be functionally characterised, their transcriptional changes have shown strong correlation to NO_3^- uptake and HATS activity indicating a similar role to their Arabidopsis counterparts [11, 35]. In Arabidopsis, *NRT2.4* is expressed in both the epidermis of lateral roots and in shoot tissue with affinity for NO_3^- at very low levels, suggesting this protein plays a role in both the root and shoot during N starvation [18]. Finally, *NRT2.5* in Arabidopsis has been located in the epidermis and cortex of roots at the root hair zone, and, is induced under N starvation [15, 16, 36] and suppressed by NO_3^- [16, 37]. Kotur and Glass [38] suggest the AtNRT2.5 provides the bulk of the constitutive HATS capacity. In rice the orthologous gene *OsNRT2.5* (also known as *OsNRT2.3a*) is expressed predominantly in xylem parenchyma cells of the root stele and has been demonstrated to play a role in the transport of NO_3^- from root to shoot, again under low NO_3^- conditions [39]. *OsNRT2.3b* expression is in the phloem and it is suggested be involved in NO_3^- transport within the shoot and its remobilisation to the grain [40]. In both maize and wheat the *NRT2.5* orthologues also demonstrate induction under low NO_3^- conditions [11, 32], however the difference in function between the orthologues in Arabidopsis and rice suggest that the simple one to one orthologous gene relationships for this gene will not translate into a conservation of function between dicots and cereals [27].

3. The control of nitrate uptake

Knowledge of the transporters mediating NO_3^- uptake has increased substantially in the past 30 years, however to truly understand the NO_3^- uptake system in plants the regulatory system controlling the transporter function must be elucidated. Improvements of NO_3^- uptake and NUE in crops through manipulation of NO_3^- transporters has recently been successful [29, 40], however it stands to reason that further improvements will require more complete knowledge of the regulatory system to maximise efficiency gains. There is evidence to suggest that NO_3^- uptake is controlled at the transcriptional, translational and post-translational levels. Isolation of mutants impaired in NO_3^- uptake has provided some new players in the regulatory system, however the advent of technology capacities such as systems biology has accelerated the identification of 'master regulators' or 'hub genes' which control NO_3^- uptake [41].

3.1 Transcriptional control

Transcriptional control of NO_3^- uptake is well documented. When Arabidopsis and barley plants are subjected to NO_3^- starvation and resupply, the observed changes in transcript levels of *NRT2.1* and *NRT2.2* follow changes in HATS NO_3^- uptake capacity [16, 42-48]. Mutant analyses of these genes have confirmed that they are indeed the major drivers of the changes in NO_3^- uptake capacity supporting the link between *NRT2* transcription and uptake capacity [34, 37, 49, 50]. Longer term lifecycle analysis has also shown distinct correlation between the changes NO_3^- uptake capacity changes and transcript levels of the *NRT2s* across the lifecycle of maize [11]. In Arabidopsis, maize and wheat transcript levels of some *NRT2s* have been shown to increase in response to reduction in N availability, aligning with an observed increase in NO_3^- uptake capacity [16, 28, 36].

Transcription factors (TFs) act as master switches for regulatory networks [51-53]. The first TF identified to play a role in NO_3^--responsive signalling in plants was a MADS box TF, ANR1, which regulates the proliferation of lateral roots in response to NO_3^- [54], but also exists in the signalling pathway of the 'transceptor' NRT1.1 [26]. Several members of the NIN-like protein (NLP) family of TFs, including NLP6, NLP7 and NLP8 regulate numerous genes in the NO_3^- uptake and signalling pathways including *NRT1.1*, *NRT2.1* and *NRT2.2* [55-57]. Along with regulating expression of NO_3^- related genes under a wide range of NO_3^--provision, the NLPs regulate other plant processes which indicates they likely exist at a high level in the NO_3^- uptake regulatory pathway and even co-ordinate NO_3^- uptake with related processes [58]. TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1-20 (TCP20) is involved in lateral root regulation in response to NO_3^- availability [59, 60], and was recently identified as co-regulating several NO_3^- assimilatory genes along with the NLPs [61]. NITRATE REGULATORY

GENE2 (NRG2) is another TF which interacts with NLP7, however NRG2 regulates the NO_3^- uptake and assimilation pathway differently to NLP7 indicating the complexity of the regulatory system response to NO_3^- provision requires several high-level controllers [62]. The LATERAL ORGAN BOUNDARY DOMAIN TFs LBD37, LBD38 and LBD39 are all strongly upregulated by NO_3^- provision and subsequent analysis of the mutants revealed the three TFs repress several NO_3^- uptake and assimilation genes leading to altered N phenotypes [63]. Several NUCLEAR FACTOR Y (NF-YA) TFs are regulated by NO_3^- provision (and microRNAs, see below) and a putative binding-site exists within the *NRT2.1* promoter suggesting this may be a mechanism for regulation of NO_3^- uptake [64]. Finally, HIGH NITROGEN INSENSITIVE 9 (HNI9), a chromatin modification factor, has been shown to repress activity of several cis-elements in the *NRT2.1* promoter, thereby regulating expression of *NRT2.1* along with several hundred other N-responsive genes in roots [65].

Discovery of the regulatory network controlling the NO_3^- uptake system has been accelerated by development of bioinformatic tools and associated databases and computing power. Systems biology approaches, where regulatory networks are developed *in-silico*, have allowed the discovery of putative 'hub genes' which are high-level controllers of NO_3^- uptake and assimilation [41]. These hypotheses can then be tested by manipulating the hub genes *in planta* and measuring the effect on the network. This allows identification of targets for improvement of NO_3^- uptake and also is an iterative process which strengthens the network structure for future efforts to identify the targets for manipulation. Comparison of the transcriptional responses of Arabidopsis to organic and inorganic N sources along with network analysis of the resulting gene lists identified a link between the circadian clock regulator, CCA1, and downstream responses of N-assimilation system [66]. A putative hub-gene in this network is the TF bZIP1, subsequently shown to play an important role in N-signalling response in Arabidopsis [67, 68], thereby demonstrating the validity of this approach. Modelling the transcriptional response of roots to NO_3^- provision over time allowed prediction of hub genes, such as *SQUAMOSA PROMOTER BINDING-LIKE9* (*SPL9*), which regulate a network which responds very quickly to NO_3^-, preparing the plants for longer term adaptation to nutritional status [69]. Further analysis of this time-responsive network revealed two homologous TFs, hypersensitive to low Pi-elicited primary root shortening 1 (HRS1) and HRS1 homologue 1 (HHO1), act to regulate root growth under P deficiency, but only when NO_3^- is present, indicating these TFs are a regulatory link mediating root responses to availability of multiple nutrients [70]. Transcriptional analysis of an auxin receptor mutant, *afb3*, previously identified to play a role in NO_3^--responsive root growth [71], led to development of a network model which identified a NAM/ATAF/CUC TF, NAC4, which acts downstream of AFB3 mediating root response to NO_3^- [72]. A meta-analysis of previously constructed NO_3^--responsive genetic networks identified the bZIP TFs, TGA1 and TGA4, as potential regulators of Arabidopsis response to NO_3^- provision [73]. Subsequent transcriptional analysis of the *tga* mutants revealed that the TFs directly regulate *NRT2.1* and *NRT2.2* transcription, but also regulate root growth responses to NO_3^- provision [73]. Another meta-analysis approach using a machine learning algorithm known as discriminative local subspaces identified the Bric-a-Brac/Tramtrack/Broad TFs, BT1 and BT2, as hubs in regulating plant response to NO_3^- [74]. Analysis of the mutants in Arabidopsis indicated that the TFs do regulate sub-traits determining NUE, including through control over several *NRT2* genes, and this regulation was shown to exist for the orthologues in rice demonstrating the suitability of Arabidopsis as a model for studying regulatory networks in more genetically complex plants like cereals [74, 75].

Commonly, TFs elicit their control by interacting with cis-acting elements and/or with other transcription factors to control gene expression [51-53]. To date, identifying NO_3^--specific cis-trans regulatory elements has focused heavily on finding NO_3^--responsive cis-elements (NREs) involved in triggering the NO_3^- -inducible expression associated with the primary NO_3^- response (PNR). The promoter regions of the NO_3^- reductase genes (*NIA1* & *NIA2*) have been extensively studied in Arabidopsis and spinach revealing a number of key cis-elements with the ability to drive NO_3^- induced expression in minimal promoter studies [76-79]. For the *NRTs*, the Arabidopsis *AtNRT2.1* promoter has been analysed using a minimal promoter approach which identified a 150 bp sequence required for the gene's NO_3^- expression and N metabolite repression responses [80].

Deletion analysis of the rice *OsNAR2.1* (Os*NRT3.1* – see below) promoter identified a 311 bp region necessary for the NO_3^- responsive transcriptional activation of the gene [81]. Subsequent motif analysis of that sequence revealed three putative NO_3^--responsive cis-elements which had all previously been associated with the NO_3^- responsiveness of the *NIA* genes in Arabidopsis and spinach: 5'-GATA-3' [79, 82], 5'-A(c/G)TCA-3' [76], and 5'-GACtCTTN10AAG-3' [77, 78].

3.2 Post Transcriptional

Micro RNAs (miRNAs) have emerged as another mode of master regulation governing gene expression in plants [83, 84]. Many studies have now revealed that miRNAs can regulate plant adaptive responses to nutrient deprivation [85-90]. Significant differences in miRNA accumulation have been observed in response to NO_3^- availability, especially under low NO_3^- conditions [91-93]. The repression of six miRNAs (miR528a/b, miR528a*/b*, miR169i/j/k, miR169i*/j*/k*) in maize roots in response to prolonged low NO_3^- provision has been suggested to play a key role in integrating NO_3^- signals into root developmental changes [94]. The small RNA miR167 has been shown to mediate lateral root initiation and growth in response to NO_3^- in Arabidopsis, putatively through regulation of the TF ARF8 [95]. Pant et al [88] found several NO_3^- responsive miRNAs in Arabidopsis and different members of the miR169 family have been shown to be involved in the long distance signaling that regulates NO_3^- starvation responses [64]. The NO_3^- induced miR393 was identified in a transcriptomics study and shown to target an auxin receptor *AFB3*, revealing an N-responsive module that controls root system architecture in response to external and internal N availability in Arabidopsis [71]. Compared to modifying transcriptional and post-transcriptional activation, it is anticipated that miRNA transcription and processing may be less energy intensive [96]. Subsequently it has recently been proposed that modification of miRNAs may be an attractive option for improving NUE in plants [96]. However, at this stage no miRNAs have been shown to specifically target and regulate the *NRT*s. With that said, given the increasing research interest in this area it appears likely that it may only be a matter of time until *NRT* specific miRNAs are identified which would open new opportunities for improving N uptake efficiency (NupE) for improved NUE in cereals.

3.3 Post translational

Post-translational regulation has also been demonstrated as an important mechanism controlling NO_3^- uptake and assimilation [97-99]. The post-translational control of NR activity is well characterised. The NR enzyme is inactivated by a two-step process involving the phosphorylation of Ser residue 543, followed by the inhibitory binding of a 14-3-3 protein kinase (see review by [100]). Focusing on the NRTs, AtNRT1.1 (CHL1/NPF6.3) has been demonstrated as a dual affinity transporter under post-translational control. When AtNRT1.1 is phosphorylated at T101 by the calcineurin B-like (CBL)-interaction protein kinase CIPK23, AtNRT1.1 functions as a high affinity NO_3^- transporter and when T101 is dephosphorylated it functions as a low-affinity NO_3^- transporter [22-25]. Phosphorylation status of AtNRT1.1 also determines the affinity for transport of auxin, a function associated with its role as a 'transceptor' thereby mediating NO_3^- uptake and regulating lateral root development in response to NO_3^- provision [101]. Further upstream of this interaction is CBL9, which plays a role in determining the affinity of AtNRT1.1 and the downstream genes regulated by this signalling pathway [25]. A number of conserved protein kinase C recognition motifs have been identified in the N- and C-terminal domains of NRT2.1 [102] suggesting that phosphorylation events may be involved in regulating NRT2.1 activity as has been demonstrated for NRT1.1. Subsequent analysis has shown that Ser28 is phosphorylated in low NO_3^- conditions and is rapidly dephosphorylated by high NO_3^- treatment, suggesting post-translational modification of NRT2.1 is important for adaptation of NO_3^- uptake capacity to changing NO_3^- provision [103].

Most notably, the AtNAR2.1 (AtNRT3.1) protein has been shown to constitute part of a two-component NO_3^- HATS system which is essential for high affinity NO_3^- transport [104]. The AtNAR2.1 protein is not a transporter itself but is a partner protein which has been shown to interact with AtNRT2.1 on a protein level at the plasma membrane [105]. Subsequently it has been shown that AtNRT2.1 may only function when in a complex with AtNAR2.1 in the plasma membrane, and may exist as a heterotetramer consisting of two subunits each of AtNRT2.1 and AtNAR2.1 [106]. It is tempting to speculate that this interaction in the plasma membrane and putative involvement of the membrane trafficking system may be important for regulating this interaction, thus providing a quick response method of adapting plant NO_3^- uptake capacity to changes in NO_3^- provision. This would be an analogous system to the one controlling Fe uptake in plants which is regulated by the trafficking of membrane transporters to the plasma membrane in combination with the absolute amount of the transporter transcript or protein present [107]. In Arabidopsis, all NRT2s with the exception of AtNRT2.7 appear to require interaction with AtNAR2.1 to facilitate NO_3^- transport [108]. This two component NO_3^- uptake system has also been shown to hold true in barley (*Hordeum vulgare*) and rice (*Oryza sativa*) for orthologous NRT2 and NAR2.1 proteins [81, 109]. Interestingly, only one of the two splice variants of the rice OsNRT2.3 (an orthologue of AtNRT2.5) requires interaction with OsNAR2.1 to mediate NO_3^- uptake [110, 111]. OsNRT2.3b has a 30 amino acid deletion and suggests this region may be important for interaction with OsNAR2.1 as is the case for OsNRT2.3a. However, when *OsNRT2.3b* is overexpressed in rice it provides an increase in NO_3^- uptake and improves NUE of the transformed plants compared to wild-type, a result that is not obtained in plants overexpressing *OsNRT2.3a* [40]. Together this information highlights the influence of post-translational control mechanisms on the NO_3^- uptake system.

3.4 Signalling

There has been a significant amount of work attempting to unravel what molecules act as signals for communicating NO_3^- supply and demand to trigger changes in the plants NO_3^- uptake system. Nitrate itself has been shown to act as a signal molecule that regulates its own uptake [102, 112, 113] which is a property not shared by other ions and their associated transport systems. Reduced nitrogen sources have also been shown to regulate NO_3^- uptake with NH_4^+ inducing strong inhibitory effects on NO_3^- uptake [114]. Supplying amino acids as the sole nitrogen source exerts strong inhibition on NO_3^- uptake [115]. Individual amino acid levels, particularly glutamate and glutamine, have been strongly linked to gene expression and feedback repression of genes involved in NO_3^- uptake and assimilation [47, 48]. To date no one metabolite has been identified as the key signalling molecule regulating the NO_3^- uptake system and this remains a key area of interest amongst the scientific community.

Recent work has identified a role for Ca^{2+} as a signalling intermediate in regulating NO_3^--responsive gene expression responses [116]. Nitrate elicits a rise in cytoplasmic Ca^{2+} levels as detected by lines expressing the Ca^{2+} reporter, aequorin. The response was not detected in lines which were treated with $LaCl_3$, a Ca^{2+} channel blocker, or EGTA, a chelating agent. The Ca^{2+} response did not occur in *NRT1.1* mutants, indicating the response requires the 'transceptor' function of that protein to elicit a response. The NO_3^- treatment also elicits an increase in IP3 (1, 4, 5-triphosphate) suggesting that the activity of a phospholipase C (PLC), the enzyme which generates lipid secondary messengers, is required in this response. Importantly this response was not observed in plants treated with the PLC inhibitor, U73122, and there was no transcriptional response of *NRT*s when treated with NO_3^-.

The role of Ca^{2+} as an intermediate has been identified in another NO_3^- induced signalling pathway [117]. Nitrate triggers a unique and dynamic Ca^{2+} signature in the nucleus and cytosol which activates the subgroup III Ca^{2+}-sensor protein kinases, CPK10, CPK30 and CPK32. These kinases in turn regulate many of the genes involved in the primary NO_3^- response including *NRT2.1*, *NRT2.2* and *NRT3.1*. However, the kinases also regulate the transcription factor, NLP7, which has been shown to be a master regulator of the primary NO_3^- response. Thus, this signalling pathway regulates NO_3^- uptake and assimilation as well as growth responses to N availability.

CIPK8 has also been shown to mediate NO_3^- sensing and to positively regulate the NO_3^--induced expression of PNR associated genes including *NRT1.1* (CHL1/NPF6.3), *NRT2.1* and *NRT2.2* [118]. It is likely that this kinase causes posttranslational modifications to protein(s) related to NO_3^- uptake, however the identity of the target protein(s) is currently unknown.

An elegant study uncovered the role of a mobile transcription factor, ELONGATED HYPOCOTYL5 (HY5) [119], in regulating the NO_3^--induced signalling pathway. Illumination of the shoots of Arabidopsis plants caused upregulation of *HY5* and subsequent transport to the root through phloem [120]. Once HY5 reaches the root it elicits an upregulation of *NRT2.1* thereby increasing uptake of NO_3^-. The complex interaction between light and N signalling pathways are linked by HY5 and further work is required to disentangle these pathways to determine how to manipulate higher level regulators to improve plant responses to changing light or N availability.

Small peptides play a role in signalling of N status in plants. CLAVATA3/Endosperm surrounding region-related (CLE) peptides are induced by N deficiency are perceived as part of a signalling module with the CLV receptor together regulating lateral root development [121]. C-terminally encoded peptides (CEPs) have been demonstrated to be part of the long-distance signalling pathway informing the shoot of the availability of N supply by the roots through the xylem and are detected by leucine-rich receptor kinases in the shoot, CEPR1 and CEPR2 [122]. Subsequently, the class III glutaredoxin polypeptides CEP DOWNSTREAM 1 (CEPD1) and CEPD2 are produced and have been found to be upregulated in the shoot in response to N deficiency and move to the root through phloem where they induce upregulation of *NRT2.1* [123].

The plant hormones cytokinin, abcisic acid and auxin have all been linked to N-status signalling pathways. Cytokinin increases in roots treated with NO_3^- through an induction of the *IPT3* gene, mediated by NRT1.1 (NPF6.3) and this cytokinin can serve as a signal to shoots of NO_3^- availability [124]. However, cytokinin also acts as a signal from shoots of N status as suggested by the loss of systemic N signalling in *IPT* mutant lines [125]. ABA regulates ABI2, a phosphatase induced by ABA, and together are part of signalling pathway along with NRT1.1 (NPF6.3) which regulates NRT2.1 [126].

4. Conclusions

We know a considerable amount about the uptake of NO_3^- and its regulation in Arabidopsis. In terms of progressing towards the development of cereal crops with high NUpE we have identified three main knowledge gaps.

4.1 The uptake systems and signalling molecules

As highlighted previously the accepted paradigm describing the LATS and HATS contribution to total NO_3^- uptake in Arabidopsis has recently been challenged by showing that the HATS is also responsive to N demand at high NO_3^- concentrations and appears to be responsible for a major proportion of the NO_3^- uptake capacity in cereals [11, 12]. Resolving the ambiguity around the contribution of each system to NO_3^- uptake in cereals is important for focusing NUpE improvement efforts on specific NRT transporters and revealing the signals modulating the NO_3^- uptake system in response to NO_3^- supply and demand.

4.2 Leveraging the PNR literature

The majority of the literature regarding NO_3^- uptake focused around PNR NO_3^- starvation and re-supply experiments in Arabidopsis [127]. It is important to understand how the results stimulated by this perturbation relates to NO_3^- uptake in the context of improving NUpE in cereals, i.e. more realistic N demand and supply. Understanding the relationships between these experimental models could provide key insight into the complex regulation networks governing the NO_3^- uptake system.

4.3 New leads for transcriptional control

With such a core role in all aspects of plant function there is evidence that TFs have played a major role in crop improvement over the years of crop domestication and breeding [128-130]. Consequently, TFs have been suggested as attractive candidates for engineering complex traits such as NUpE and NUE [131, 132]. As highlighted previously, with evidence of such strong transcriptional control over the *NRTs* there is the potential to exploit key cis-trans regulatory elements to increase functional NRT levels for improved NUpE. Therefore, discovery of novel *NRT* cis-trans regulatory elements and determination of whether regulatory mechanisms discovered in Arabidopsis exist in cereals appears to be an attractive step to enable the production of cereals with increased NUpE and overall improved NUE.

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Figure Captions

Fig. 1: Summary of key transporters and regulators mediating NO_3^- uptake in plant roots. The low-affinity transporter, NRT1.1 and the high-affinity transporters, NRT2.1 and NRT2.2 are involved in acquiring NO_3^- from the rhizosphere, while NRT2.5 mediates the loading of NO_3^- into the transpirational stream in the stele. The root tissue types represented are: epidermis (EP), cortex (CO), stele (ST). Depicted are the transporters (circles), transcription factors (squares), kinases (trapezoids), peptides (triangles) and chromatin regulators (pentagons). Regulation of the transporters which has been established as direct interaction (red arrows) or indirect interaction (or not determined to date) (blue arrows). Transporters are localised to the tissue in which they are most highly expressed, and the area of the transporter circle represents the relative expression level of the genes encoding the respective transporter in either low (left) or high (right) NO_3^- provision. Top half of the diagram represents mature root tissue, while the bottom represents the root tip region.

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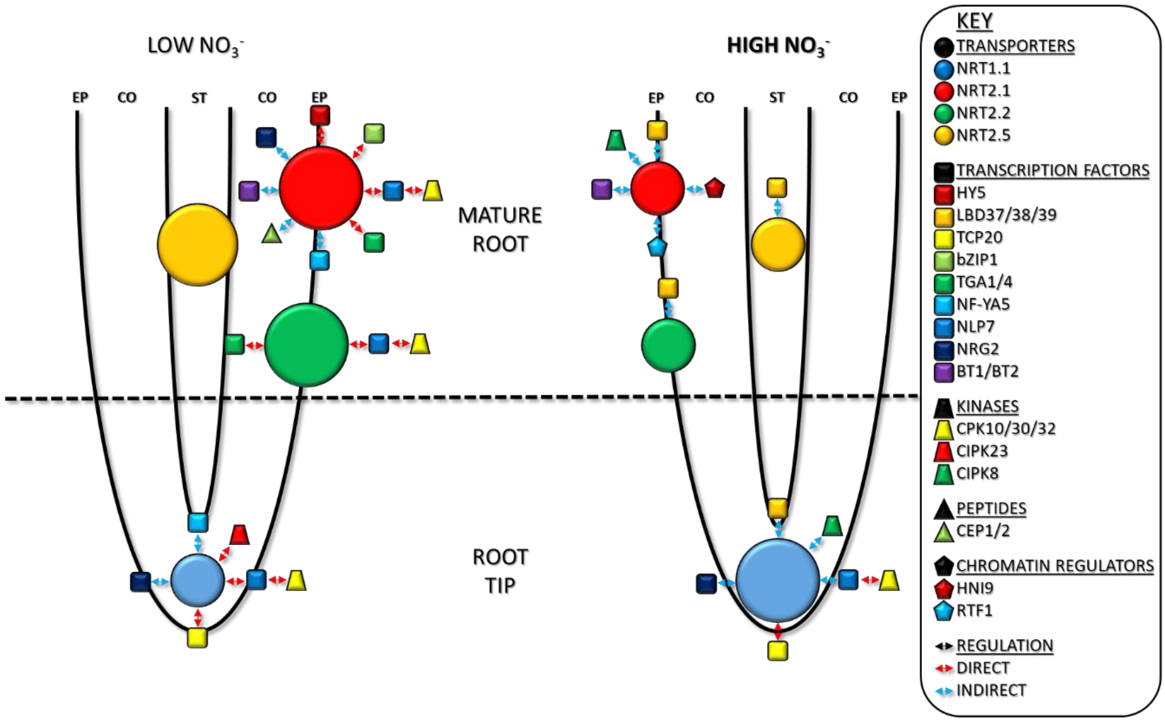

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