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Review

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.

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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 improving 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

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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]. 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 [27], 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 [28,29].

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 is constitutively expressed [30]. 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. 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 [31], 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 [32]. 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 [17,33]. 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 [11,34]. 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 [15,16,35] and suppressed by NO_3^- [16,36]. Kotur and Glass [37] 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 [38]. *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 [39]. In both maize and wheat the *NRT2.5* orthologues also demonstrate induction under low NO_3^- conditions [11,31], 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.

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 [28,39], 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 [40] (Fig. 1).

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 [16,41–47]. 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 [33,36,48,49]. Longer term lifecycle analysis has also shown distinct correlation between the changes NO_3^- uptake capacity changes and transcript levels of the *NRT2 s* across the lifecycle of maize [11]. In Arabidopsis, maize and wheat transcript levels of some *NRT2 s* have been shown to increase in response to reduction in N availability, aligning with an observed increase in NO_3^- uptake capacity [16,27,35].

Transcription factors (TFs) act as master switches for regulatory networks [50–52]. The first TF identified to play a role in NO_3^--responsive signalling in plants was a MADS box TF, ANR1,