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Title

Post-anthesis nitrate uptake is critical to yield and grain protein content in *Sorghum bicolor*

Authors

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Abstract

Crops only use ~50% of applied nitrogen (N) fertilizer creating N losses and pollution. Plants need to efficiently uptake and utilize N to meet growing global food demands. Here we investigate how the supply and timing of nitrate affects N status and yield in *Sorghum bicolor* (sorghum). Sorghum was grown in pots with either 10 mM (High) or 1 mM (Low) nitrate supply. Shortly before anthesis the nitrate supply was either maintained, increased 10-fold or eliminated. Leaf sheaths of sorghum grown with High nitrate accumulated nitrate in concentrations >3-times higher than leaves. Removal of nitrate supply pre-anthesis resulted in the rapid reduction of stored nitrate in all organs. Plants receiving a 10-fold increase in nitrate supply pre-anthesis achieved similar grain yield and protein content and 29% larger grains than those maintained on High nitrate, despite receiving 24% less nitrate over the whole growth period. In sorghum, plant available N is important throughout development, particularly anthesis and grain filling, for grain yield and grain protein content. Nitrate accumulation in leaf sheaths presents opportunities for the genetic analysis of mechanisms behind nitrate storage and remobilization in sorghum to improve N use efficiency.

Keywords

nitrogen, nitrate, nitrate accumulation, nitrogen remobilization

1. Introduction

Nitrogen (N) is critical for plant growth, particularly for production in high-yielding crop systems. Synthetic N fertilizers are generated by fixing atmospheric N₂ into plant available inorganic N forms, via the energetically expensive Haber-Bosch process, and have contributed to increasing crop yields over the last century (Erisman et al., 2008). The economic and environmental impacts of N fertilizers are the impetus for developing better management strategies for N fertilizer application in conjunction with the breeding of crops with improved N uptake and utilization (Robertson and Vitousek, 2009). Synthetic N fertilizers are readily converted to nitrate via microbial activity and result in nitrate often being the most plant-available N form in agricultural soils (Crawford
and Glass, 1998; Robertson and Vitousek, 2009). Due to its high mobility and reactivity, nitrate is considered to be responsible for much of the N pollution associated with cropping activity (Robertson and Vitousek, 2009). Global and local N pollution can take the form of toxic nitrate-laden water bodies, hypoxic zones, acidic soils and increases in atmospheric nitrous oxide (Davidson, 2009; Gu et al., 2013; Robertson and Vitousek, 2009; Tilman et al., 2002; Turner et al., 2008). Manipulating nitrate supply in controlled conditions is an avenue to understanding how crops acquire, store and remobilize nitrate to achieve and maintain maximum productivity whilst minimizing the loss of reactive N to the environment.

Regardless of its form, when plant-available N is in plentiful supply, some plants take up and store nitrate, even though it is eventually more energetically expensive to assimilate than ammonium (Meyer and Stitt, 2001). After root uptake and translocation, nitrate is mainly assimilated in the shoot by nitrate reductase followed by nitrite reductase to produce ammonium (Andrews et al., 2013). Nitrate is stored in the vacuole whereas the reduction of nitrate and nitrite is believed to occur in the cytoplasm and chloroplast, respectively (Krapp, 2015; Meyer and Stitt, 2001). However, utilizing nitrate as the primary N source appears to be an evolutionarily derived trait characterizing and differentiating modern plant species because some plants have a limited capacity to acquire or store nitrate (Stewart and Schmidt, 1999). Contrary to ammonium and its potential cumulative toxicity, nitrate has the advantage of being able to be stored without the requirement for immediate assimilation (Britto and Kronzucker, 2002; Meyer and Stitt, 2001; von Wirén et al., 2001). Model plant *Arabidopsis thaliana* (Arabidopsis) has the highest concentrations of nitrate in the petiole, as do other small vegetative dicotyledonous plants (Chiu et al., 2004; Santamaria et al., 1999). It is believed the accumulation of nitrate in cell vacuoles may be an advantageous trait in crop breeding, enabling surplus uptake of nitrate when N availability is high, for use later in the plant’s lifecycle (Hirel et al., 2007). Relatively higher grain yields have been achieved in genotypes of *Zea mays* (maize) that accumulate more nitrate in early plant development, presumably because the stored nitrate is remobilised and assimilated during grain fill (Hirel et al., 2001). Similarly, *Sorghum bicolor* (sorghum), a related C4 grain crop of the Panicoideae subfamily of Poaceae, can accumulate nitrate in young shoots (Gleadow et al., 2016; Robinson et al., 2011). However, there is minimal information on nitrate accumulation in cereal crops, how stored nitrate impacts the N status over the plant’s lifecycle, and ultimately, how nitrate stores could influence yield and grain protein. The molecular physiology of nitrate is comparatively well-studied and reviewed in Arabidopsis with 24 genes within four families of diverse transporters characterized to facilitate nitrate transport (Krapp et al., 2014). In contrast, relatively few studies have been undertaken to investigate nitrate transporter gene orthologues in grain crops and, unsurprisingly, gene expression can vary from Arabidopsis in tissue localization and response to N supply (Buchner and Hawkesford, 2014; Garnett et al., 2013; Plett et al., 2010). In wheat, Buchner and Hawkesford (2014) identified 16 nitrate transporter gene orthologues expressed in root and shoot under variable nitrate conditions, concluding cereal crops have more complex gene expression and regulatory patterns than Arabidopsis. A natural variant of a root expressed nitrate transporter in rice is, in part, responsible for improved growth and yield under low N supply (Hu et al., 2015). A further two transporters in rice are implicated in nitrate uptake and translocation with the potential to improve nitrogen use efficiency (NUE) in the future (Li et al., 2015; Xia et al., 2015). The complexity of cereal crop gene expression has been explored in maize using hydroponics, finding expression and nitrate uptake highly variable at across the lifecycle, despite a constant nitrate supply (Garnett et al., 2013).
Whilst there are numerous studies on plentiful and limiting N on the growth and yield of cereal crops and how remobilized N contributes to grain protein, it is not clear where, when and how nitrate is stored and utilized in cereal crops. In this study, we hypothesize the primary location of nitrate storage in sorghum is the leaf sheath, anatomically analogous to the petiole. To improve current knowledge, we will investigate the changes in nitrate storage over sorghum’s lifecycle, under four variable nitrate supply regimes, and examine how nitrate supply affects vegetative growth, flowering and grain fill. Our aim is to identify the primary location(s) of nitrate storage and the key developmental stage(s) of shifts in nitrate storage to enable future work in the identification of the genes and pathways involved in nitrate storage and remobilization in sorghum.

2. Materials and methods

2.1 Plant material, growth conditions and nitrate treatments

The plant material used was the inbred line R931945-2-2 developed by the Queensland Department of Agriculture and Fisheries sorghum breeding program. Three sorghum seeds were planted per 200 mm diameter (4 litre) ANOVApot® (Anova Solutions Pty Ltd, Australia) and saucer filled with an inert growth medium (medium grade double-washed river sand pH7.1). Seedlings were thinned to one per pot prior to commencing nutrient application, nine days after sowing (DAS). Each pot received 100 mL of nutrient solution three days per week containing: 2 mM MgSO4; 2 mM CaSO4; 0.457 mM KH2PO4; 42.5 μM K2HPO4; 100 μM FeEDTA; 10 μM MnSO4; 10 μM H3BO3; 1 μM CuSO4; 2.5 μM ZnSO4; and 0.35 μM Na2MoO4 (Robinson et al., 2011). One of two nitrate treatments either 1mM KNO3 (+ 4.5 mM K2SO4; Low) or 10 mM KNO3 (High) complemented the basic nutrient solution. Nitrate was the only N source in the nutrient solutions to eliminate confounding factors such as other N compounds acting as a signal (Cerezo et al., 2001; Tsay et al., 1993). Nitrogen supplied at 10 mM is considered replete for this type of experiment (Migocka et al., 2013; Robinson et al., 2011) and the limiting N supply was set 10-fold lower to induce a differential growth response. After harvest 1 at 55 DAS, each pot was flushed with 1,000 mL of tap water and left to drain. The tap water and final leachate were tested to ensure they did not contain residual nitrate (MQuant™ Nitrate Test strips, EMD Millipore Corporation, MA, USA). Half the remaining plants were maintained on the original nitrate treatment of either 1 mM (Low-Low) or 10 mM (High-High) nitrate supply. The other plants, originally receiving 10 mM KNO3, now received zero nitrate (+5 mM K2SO4; High-Zero) to procure a rapid response from the elimination of nitrate supply. The remaining plants, originally receiving 1 mM KNO3, now received 10 mM KNO3 nitrate (Low-High). Nitrate treatments and harvest timeline are summarized in Fig 1. Plants were grown in a naturally lit glasshouse in Brisbane, Australia from June to October in a randomized design of each nitrate treatment. The glasshouse temperature ranged from 18°C to 30°C and relative humidity ranged from 72% to 87%. Each treatment and harvest contained three independent replicate pots with one plant per pot, except for the High-Zero treatment at 133 DAS (grain maturity) with two replicates.

2.2 Harvest

At approximately one week prior to flag leaf stage, 55 DAS, three plants from each of the two nitrate treatments (High and Low) were harvested between 10:00 and 12:00 in the morning. Each plant was separated into: (1) leaves; (2) leaf sheaths; (3) immature leaves/inflorescence; and (4) roots. The roots were washed to remove sand. Plant samples were oven dried at 55°C for five days and weighed. Dried plant samples were finely ground (Retsch ball mill MM400, Hahn, Germany). Nitrate content was determined on 20% methanol dried plant sample extracts.
as described by Miranda et al. (2001). Nitrogen content of dried plant samples was determined by dry combustion and infrared detection in a LECO analyser (CNS-2000, LECO Corporation, MI, USA).

2.3 Subsequent harvests

Plants from all four nitrate treatments were harvested an additional four times, up to and including maturity. Four and eight days after the nitrate supply regime was changed (refer 2.1) plants were harvested, 59 and 63 DAS, as described for harvest 1. Plants were also harvested at anthesis (76-86 DAS as the nitrate treatment induced developmental variation) and at grain maturity (133 DAS). Due to the different stages of development, i.e. vegetative, anthesis and grain maturity, the samples taken in the latter two harvests differed and were separated into: (1) flag leaf; (2) other leaves; (3) stem, including leaf sheaths and peduncle; (4) roots; and (5) inflorescence or mature grain head.

2.4 Yield components and nitrogen use calculations

Grains were removed from the spikelet, hulled and weighed to determine yield per plant. Grains were manually counted three times for grain number. Protein content of grain was estimated using the cereal N-to-protein conversion factor, N% grain yield x 5.6 (Mariotti et al., 2008). Nitrogen harvest index, NHI = grain N (mg) / shoot N (mg) x 100, and is the total grain N content expressed as a percentage of the total shoot N content (Fageria, 2014). Physiological efficiency, PE = (grain weight treatment – grain weight control) / (shoot N treatment – shoot N control), is the change in grain weight expressed as a proportion of the change in shoot N content, where the mean values of Low-Low plants functioned as the control (Craswell and Godwin, 1984; Garnett et al., 2015).

2.5 Statistical analysis

STATISTICA v.13 (Dell, Inc.) was used to determine statistical significance (p < 0.05) with two-way ANOVA and Tukey’s HSD post hoc. Biomass, nitrogen concentration and nitrate concentration data were log10-transformed prior to statistical analysis.

3. Results

3.1 Growth and yield components

Plants displayed a differential growth response to nitrate supply and by 55 DAS those raised on a 10 mM (High) nitrate supply were almost double the biomass of those raised on a 1 mM (Low) nitrate supply (Fig 2a). At developmental maturity (133 DAS) whole plant biomass of High-High plants (54.79 ± 2.77g) was 3.5-times that of Low-Low plants (15.42 ± 0.31g; Fig 2a). Growth of High-Zero plants had stagnated by anthesis (76 DAS), and mature whole plant biomass was 35.88 ± 3.39g, one-third smaller than High-High plants (Fig 2a). However, Low-High plants recovered from the initial limiting nitrate supply and enhanced growth resulted in mature whole plant biomass of 47.60 ± 1.85g, only 13% smaller than High-High plants (Fig 2a). The inflorescence biomass of High-High and High-Zero plants was similar but subsequent grain yield of High-Zero plants was 65% lower than High-High plants (p < 0.01; Table 1; Suppl. Table S1). Conversely, despite the inflorescence biomass of Low-High plants being 61% smaller than High-High plants (p < 0.01), the resulting yield was only 16% lower and not significantly different (Table 1; Suppl. Table S1).

Grain number of Low-High plants was 5-times greater than Low-Low plants, but 35% fewer than High-High plants (p < 0.01; Table 1). Low-High plants had a grain yield of 11.35 ± 0.63g but with fewer grains compared to
High-High plants (13.48 ± 1.08g), therefore, the Low-High plants produced seeds 28% larger than High-High plants (p < 0.005; Table 1). Detailed biomass data can be found in Suppl. Table S1.

The estimated grain protein content was not significantly different between Low-High and High-High plants, 11.1 and 9.9%, respectively (Table 1). To summarize, the Low-High plants produced larger seeds with the same protein content with significantly lower leaf biomass throughout development (p < 0.05) and 24% less total N supply than High-High plants (Table 1; Suppl. Table S1).

3.2 Nitrogen utilization

The physiological efficiency (PE) and nitrogen harvest index (NHI) of High-High and Low-High plants was significantly higher (p < 0.05) than High-Zero and Low-Low plants (Table 1).

Post-anthesis N uptake (PANU) represented 37 and 38% of total plant N in High-High and Low-Low plants, respectively, however PANU in Low-High plants was 62% of total plant N (Fig 2b). Nitrogen uptake did not mirror biomass accumulation and thus whole plant N concentration declined leading up to anthesis, remaining consistent at grain maturity (Fig 2c). Detailed N concentration data can be found in Suppl. Table S2.

3.3 Nitrate storage

Minimal nitrate concentrations were detected in Low plants at 55 DAS (Fig 3) but eight days after receiving an increased supply of nitrate the Low-High plants contained low nitrate concentrations in all organs, up to 1 mg NO$_3^-$ g$^{-1}$ dw, except for leaves where no nitrate was detected (Fig 4). At 55 DAS, High plants had accumulated nitrate in all organs at concentrations between 6 and 33 mg NO$_3^-$ g$^{-1}$ dw with leaf sheaths’ nitrate concentrations up to 4-times that of other plant parts (Fig 3). Leaves and leaf sheaths of High-Zero plants rapidly lost stored nitrate four and eight days after nitrate supply was eliminated, with nitrate concentration decreasing by 30 and 85%, respectively (Fig 4a & b). Nitrate concentrations in roots and immature leaves/inflorescence of High-Zero plants also decreased by 52 and 77%, respectively, after four days of nitrate deprivation (Fig 4c & d). Despite High-High plants being maintained on 10 mM nitrate supply, nitrate stores in all organs had also diminished considerably by inflorescence (Fig 4a-d). At 55 DAS, nitrate-N represented 13% of total N pool in roots, 5% in leaves and 5% developing meristem. Of the plant organs analyzed here, nitrate-N made up the highest proportion of the N pool in leaf sheaths at 27%. Detailed nitrate concentration data can be found in Suppl. Table S3.

4. Discussion

This glasshouse study revealed two main findings. Firstly, early nitrate limitation followed by an enhanced nitrate supply pre-anthesis, Low-High treatment, generated a slight yield reduction of 16%, with 35% fewer but 28% larger grains. Secondly, 4-fold higher nitrate concentrations were found in sorghum leaf sheaths, compared to other plant parts, representing 27% of the leaf sheath total N pool.

4.1 Grain yield, protein content and seed size is dependent on post-anthesis nitrate uptake

The Low-High plants had fewer, bigger grains with the same protein concentration compared to High-High plants, despite Low-High plants receiving 24% less nitrate overall. In cereal crops, breeding for higher yields has led to the understanding that grain yield (GY) and grain protein content (GPC) are negatively correlated and influenced by both genotype and environment (Hirel et al., 2007; Kesavan et al., 2013; Simmonds, 1995; Sinclair, 1998). In this glasshouse experiment we manipulated the N environment by increasing nitrate supply at a critical developmental time point, pre-anthesis, and achieved similar GY and GPC with less applied nitrate than High-High plants. In high-yielding wheat cultivars, Martre et al. (2003) suggests that early grain development is dependent on sink size and N supply. However, the subsequent grain filling stage was dependent entirely on N
supply (Martre et al. 2003). In our study, the potential sink size at anthesis was not significantly different between High-High and High-Zero plants but removal of the N supply meant grain filling was impeded so High-Zero plants had a 65% yield reduction. Conversely, a significantly smaller sink size at anthesis in Low-High plants was largely compensated by the increased N supply, resulting in larger grain size that markedly improved yields. Our results support the conclusions of Martre et al. (2003) that grain filling is reliant on N supply and emphasizes the importance of N availability during grain filling.

In field-grown wheat, the negatively correlated relationship between GPC and GY is termed grain protein deviation (GPD; Bogard et al. 2010). Post-anthesis N uptake (PANU) is a major determinant of GPC, despite only contributing an average of 16% of total GPC, with the remainder coming from remobilization (Bogard et al., 2010). Glasshouse grown maize with $^{15}$N-labelled fertilizer applied at silking, a developmental time point corresponding to anthesis, found the majority (> 60%) of the late-applied $^{15}$N was in the grain, further supporting the importance of PANU for GPC (Subedi and Ma, 2005). Field studies in wheat grown with variable N supplies showed PANU can vary considerably, contributing anywhere between 10 and 50% of grain N and highlighting the influence of genotype and N supply (Gaju et al., 2014; Kichey et al., 2007). During grain filling, sorghum continues N uptake from the soil, as well as remobilizing N from the stem and leaves (Borrell and Hammer, 2000; Sinclair et al., 1997). In contrast to wheat, the PANU contribution to grain N is 49% in senescent sorghum genotypes and 64% in stay-green sorghum genotypes (Borrell and Hammer, 2000). In our study, total plant N at anthesis compared to grain maturity indicated the majority of grain N in High-High and Low-High plants is from PANU rather than remobilization, consistent with stay-green sorghum genotypes (Borrell and Hammer, 2000). The stay-green trait in sorghum delays the onset of leaf senescence allowing photosynthesis to continue during grain filling (Jordan et al., 2011). We hypothesize that the combination of delayed senescence and PANU contributes to sorghum’s plasticity and recovery after limiting N conditions, demonstrated by the yield results of our Low-High treatment. Here, the increased N supply during anthesis and grain filling (Low-High), in conjunction with the stay-green trait, may have resulted in enabling ongoing carbon fixation and nitrogen synthesis and given rise to the larger grains containing high protein, despite a 61% smaller inflorescence, compared to High-High.

Field and glasshouse experiments in sorghum and maize have shown the degree of N limitation and the timing of additional N fertilization can influence GY and GPC (Asher and Cowie, 1974; Paponov et al., 2005a; Peng et al., 2013; Subedi and Ma, 2005). Two maize genotypes with differing sensitivities to N limitation were grown at optimal N supply, where GY was the same, and sub-optimal N supply where GY was 23% lower in the N sensitive genotype (Paponov et al., 2005a). When N supply was increased at silking, GY recovered to 84 and 95% (N sensitive and high NUE genotype, respectively) compared to plants grown at optimal N supply continuously (Paponov et al., 2005a). However, if the increased N application was delayed to 14 days after silking, there was no increase in GY indicating how critical the timing of N fertilization is relative to developmental stage (Paponov et al., 2005a). Also in maize, Peng et al. (2013) found excessive N applications did not result in an increase in total N uptake or GY but concluded there was benefit to fertilizer applications being split to include the critical period from V8 to silking, a developmental period of highest N uptake. These studies corroborate the results of our study and demonstrate the importance of N availability at critical developmental time points in cereals, like sorghum and maize, to maximize GY.
A large grain size in cereal crops is a desirable trait brought about by domestication and more recently the selective breeding of QTL and genes identified to control seed size (Hancock, 2012). Our study indicates the potential to enhance seed size by manipulating the timing of N fertilization. The N sensitive maize genotype investigated by Paponov et al. (2005a) had a reduced yield at sub-optimal levels of N fertilization but kernel size remained relatively unchanged. The maize genotype with high NUE traits had a kernel size 12% smaller under optimal N fertilization and up to 26% smaller under sub-optimal fertilization, when compared to the N sensitive maize genotype (Paponov et al., 2005a). Therefore, the high NUE genotype achieved a higher GY with more kernels than the N sensitive genotype (Paponov et al., 2005a, b). In our study, Low-High plants received 24% less N resulting in a 16% yield reduction, 7% higher NHI, 29% larger seeds and similar protein content, compared to High-High plants. The evidence presented here supports the management of N fertilizer applications, especially at critical time points in development. Therefore, timing and application of N fertilizer needs to be considered alongside genotype to ensure maximal yield and grain quality in cereal crops.

4.2 Nitrate storage

Previous studies have determined when nitrate is in adequate supply, the shoots, versus the roots, of young sorghum plants contained the highest concentrations of nitrate (Gleadow et al., 2016; Robinson et al., 2011). However, shoot organs have not been differentiated to determine which contain stored nitrate and how nitrate stores fluctuate over sorghum’s lifecycle. In this study, plants receiving High nitrate supply had the highest nitrate concentrations in the leaf sheaths at 55 DAS, 4-fold higher than other organs. By anthesis, nitrate concentrations had diminished almost entirely in all organs. In grasses, the leaf sheath is analogous to the petiole of dicotyledonous plants and it is in the petiole, followed by leaves, where the highest nitrate concentrations have been reported in model plant Arabidopsis thaliana (Chiu et al., 2004) and edible leafy green vegetables (Chen et al., 2004; Santamaria et al., 1999). Direct comparisons of nitrate accumulation from other studies is difficult due to variable reporting methods, however, Chen et al. (2004) found on a fresh weight basis the nitrate concentrations of the stem were twice that of leaves in 9-week-old rape, Chinese cabbage and spinach. In sorghum, nitrate concentrations were considerably higher in younger versus older sorghum plants with 6-fold higher concentrations found in stem (primarily made up of leaf sheaths) versus leaves (Gleadow et al., 2016). Our results concur with the findings of Gleadow et al. (2016) that nitrate concentrations in sorghum shoots decrease as plants age. To our knowledge this is the first time the leaf sheaths of sorghum have been analyzed separately to assess the changing N and nitrate status under variable nitrate supplies. Previous studies have reported the leaf sheaths and leaves are the major N sinks in sorghum (van Oosterom et al., 2010b) and our study shows that, pre-anthesis, approximately 26% of leaf sheath N is nitrate and is therefore and important unassimilated N store. By identifying the leaf sheaths as the location of high nitrate concentrations in early development and determining the timing of its depletion, we have uncovered an opportunity to investigate the genetic mechanisms behind nitrate storage and remobilization at this critical time point.

4.3 The road to improving NUE in cereal crops

One of today’s agricultural challenges is to breed cereal crops with high yields and high grain quality whilst simultaneously developing sustainable agricultural systems (Hirel et al., 2001). To date, the main paths of investigation to improve crop NUE are N uptake capacity and primary N metabolism and remobilization
efficiency. The complexities of N related gene expression, regulation and metabolic pathways combined with the developmental stages and variable growing environments of cereal crops complicate and hinder investigations. The natural diversity in two sub-species of rice, *indica* and *japonica*, has a variable response to N supply (Hu et al., 2015; Sun et al., 2014). The allelic variations isolated in *DEP1 (DENSE AND ERECT PANICLES 1)* and in nitrate transporter *OsNPF6.5* contributes to an enhanced growth response and increased nitrate uptake in the *indica* sub-species (Hu et al., 2015; Sun et al., 2014). This evidence of intra-species variation in nitrate use supports the potential to uncover similar NUE variants in sorghum. The recent assessment of 230 diverse sorghum genotypes revealed nitrate transporters contained more nucleotide diversity than ammonium transporters in domesticated lines, offering the prospect of improving NUE in sorghum (Massel et al., 2016).

One alternate avenue of investigation is to increase N storage in leaves and stem by increasing N uptake in early development and enhancing N remobilization during grain fill, thereby reducing any N shortfall during grain fill. Van Oosterom et al. (2010a) suggested leaf senescence of stay-green sorghum varieties could be further delayed if the translocation of stem N could be increased during grain fill. This would be advantageous because the benefits of the stay-green trait may be at odds with maximizing grain N through remobilization as demonstrated by greater PANU in stay-green sorghum. Additional uptake and storage of nitrate may contribute to increasing the shoot N sink, delay the energetic costs of N assimilation until required thereby reducing potential N loss from the soil from early application of N fertilizer. In this study, high nitrate supply was ample to promote early vegetative growth and excess nitrate was accumulated in all parts of the young plants. Despite receiving an ongoing high nitrate supply, stored nitrate was depleted by anthesis coinciding with an increased N demand from the onset of grain development (Sinclair et al., 1997; van Oosterom et al., 2010a). A larger N store in the stem could be facilitated by a greater capacity to store nitrate and may subsequently alleviate a N supply shortfall during grain filling. Garnett et al. (2013) identified variations in gene expression of nitrate uptake genes in roots of hydroponically grown maize at different developmental stages, particularly high-affinity nitrate transporters (NRT2s) just prior to anthesis. Field studies in wheat have shown N supply, followed by developmental stage, are the biggest influencers in crop performance (Barraclough et al., 2014). Therefore, the developmental stage just prior to panicle exertion presents an ideal opportunity for examining changes in N utilization and mobilization.

5. Conclusion

Here we have identified the leaf sheath as the primary nitrate storage organ and pre-panicle exertion as a key developmental time point in sorghum. This presents an opportunity to examine key genes and metabolites involved in nitrate storage, assimilation and remobilization. Future investigations aim to uncover new targets for improving N use in sorghum through diversity analyses and transgenic studies in combination with agronomic management of N fertilizers.

Acknowledgements

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response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. New Phytol. 198(1), 82-94.


Fig. 1.
Schematic of nitrate treatments and harvest timeline: *Sorghum bicolor* plants were grown from seed for 55 days with one of two nitrate supplies and then either maintained on the same or increased nitrate supply, or had nitrate eliminated entirely, and grown to grain maturity for a further 78 days. Change in nitrate supply indicated by vertical line. Letters represent harvests: (A) harvest 1 ~ a week prior to flag leaf; (B) harvest 2; (C) harvest 3; (D) harvest 4 anthesis; and (E) harvest 5 grain maturity.

Fig. 2.
Whole plant (a) biomass (g dry weight); (b) nitrogen content (mg N); and (c) nitrogen concentration (mg N g⁻¹ dw) of *Sorghum bicolor* grown with one of four nitrate regimes. (*n* = 3 except 133 DAS for High-Zero plants where *n* = 2). Vertical line indicates change in nitrate supply. Error bars represent standard error.
Fig. 3.

Nitrate concentration (mg NO$_3^-$ g$^{-1}$ dw) at 55 DAS of *Sorghum bicolor* plants grown with high or low nitrate supply. ($n = 3$). Error bars represent standard error. Letters represent significant differences between organs within nitrate regime ($p < 0.05$).
Fig. 4.
Nitrate concentration (mg NO$_3$·g$^{-1}$ dw) of (a) leaves; (b) leaf sheaths; (c) immature leaves/inflorescence; and (d) roots of *Sorghum bicolor* over development and grown with one of four nitrate supplies. ($n = 3$ except 133 DAS for High-Zero where $n = 2$). Vertical line indicates change in nitrate supply. Error bars represent standard error.
Table 1.

Yield components and nitrogen use of *Sorghum bicolor* at grain maturity grown with one of four nitrate supplies (± standard error). NHI = nitrogen harvest index. PE = physiological efficiency (calculated in comparison to Low-Low nitrate supply). Letters indicate statistically significant differences between nitrate treatments (p < 0.05).

<table>
<thead>
<tr>
<th>N supply (mg)</th>
<th>Shoot N (mg)</th>
<th>Grain dw (g)</th>
<th>Grain N (mg)</th>
<th>NHI (%)</th>
<th>PE</th>
<th>Grain number</th>
<th>1,000 grain weight (g)</th>
<th>Grain protein (% dw)</th>
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<tr>
<td>High-High</td>
<td>952</td>
<td>405 ± 25a</td>
<td>13.48 ± 1.08a</td>
<td>238 ± 17a</td>
<td>59a</td>
<td>34a</td>
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<td>4.69 ± 0.93b</td>
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