Poor nitrogen uptake efficiency of wheat - can we fix it?

**PROJECT DETAILS**

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**Summary**

Rapid uptake of soil nitrate (NO$_3^-$) by wheat is needed to improve nitrogen (N) use for profitable and sustainable farms. The capacity of wheat to capture NO$_3^-$ was studied from nutrient solution, using glass-fronted rooting boxes to study rooting patterns, as well as using field experimentation. These methods identified differences in growth rate, the kinetics of NO$_3^-$ uptake, total N uptake and root growth. NO$_3^-$ uptake was strongly associated with early growth vigour, including leaf area development, and the production of vigorous roots with extensive branching in the top 80cm of soil. The inducible capacity of root NO$_3^-$ transporters did not appear to be important in NO$_3^-$ uptake from soil.

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Conclusions

Significant differences were detected between commercial varieties in their capacity to accumulate N under field experimentation and in controlled environment studies. However, none of the commercial wheat varieties examined exceeded the capacity of so called early vigour growth genotypes to produce dry matter and to accumulate N; most were considerably poorer in NO$_3$ capture capacity. The much larger root biomass of the vigorous wheat genotypes resulted from an early and prolific root branching in the top 0.5m of the soil rather than from deeper roots. The early and more abundant root branching in the vigorous lines increased the root length density, the number of roots and increased the capacity of wheat to uptake N compared to the commercial varieties examined. These early growth vigour genotypes do not have significantly higher NO$_3$ reductase activity, a key enzyme involved in the assimilation of N into amides and other organic N forms, when expressed on per unit fresh weight. The activity of NO$_3$ inducible NO$_3$ transporters in root tissue that are responsible for NO$_3$ transport into root cells is also either not greater in early growth vigour lines, compared to commercial wheat varieties, or the activity of such gene regulated transporters is not critical to NO$_3$ uptake under soil conditions. Therefore, on the evidence obtained to date, selection of genotypic material on the basis of the activity of NO$_3$ transport mechanisms or NO$_3$ reductase activity is not likely to lead to consistent improvements in NO$_3$ capture in wheat genotypes.

Use of novel double haploid wheat genotypes generated from crosses of two early vigour lines, CM18 and Vigour18, enabled the effect of contrasting growth traits on NO$_3$ uptake to be examined to identify phenotypic traits critical to NO$_3$ uptake in early growth vigour lines. Those with the capacity to generate rapid biomass typically possessed higher NO$_3$ uptake capacity. The importance of rapid leaf area development and presumably photosynthetic activity in NO$_3$ uptake was also identified. In contrast, higher tillering capacity in double haploid lines was poorly related to N uptake. Solution culture studies involving double haploid lines also confirmed that a wide range in NO$_3$ uptake activity exists from solutions containing the NO$_3$ concentrations in the range known to affect the activity of the NO$_3$ inducible NO$_3$ transporter in root systems. As was the case for early growth vigour lines, the kinetics of NO$_3$ induced transport in roots in double haploid lines did not appear to affect the capacity of genotypes to accumulate N when these were grown in the field. This confirmed previous findings that either superior root growth or higher demand for N induced by higher growth rates are more important determinants of N uptake from soil than root NO$_3$ transporter activity. Evaluation of the capacity of double haploid crosses of early vigour lines along with selected early vigour lines in the field showed that the capacity to accumulate N early in growth does not necessarily result in higher N uptake in the longer term, compared to commercial wheat lines, in seasons where NO$_3$ is largely retained within the top 1m of soil. Further experimentation is required to test the thesis that early growth vigour is advantageous in soils where NO$_3$ is moved to depth with leaching rainfall.

Recommendations

The consistent demonstration, both in a controlled environment, as well as in field studies, of the importance of early growth vigour and vigorous root growth in N uptake in wheat suggests that these traits should be selected for in wheat breeding programs that target regions with a high proportion of soils that are at risk of NO$_3$ leaching.
Only a small selection of the double haploid wheat lines generated in the Graingene project was appraised in this study. A more extensive examination appears to be warranted, based on the findings obtained here, with work done over several seasons or locations to evaluate the genotype by season interaction.

It is recommended that wheat genotypes be screened in the field along with root analysis. Solution studies facilitate faster screening and are easier to manage. However, genotype performance in the field was not always consistent with that observed under solution culture conditions.

The root box systems used in this study enabled a good picture of rooting behaviour to be obtained inexpensively without large expenditure of labour. However, it is recommended that methods applied, specifically the depth and bulk densities of reconstituted soil layers in the boxes, better reflect the fertility and soil physical properties encountered in the field.

The capacity of genotypes to accumulate N in the field was not strongly related to the kinetics of NO$_3^-$ induced transport in roots, shown in solution culture, suggesting that either superior root growth or higher demand for N induced by higher growth rates were more important determinants of N uptake than specific root NO$_3^-$ transporter activity. Thus, investment in studies to show differences between genotypes in genes that encode the NO$_3^-$ inducible NO$_3^-$ transporter systems would appear to present low potential returns to the wheat industry.

**Outcomes**

The project examined the capacity of commercial and experimental wheat lines to capture NO$_3^-$ and documented the importance of key physical and physiological traits in NO$_3^-$ uptake in wheat. As such, the project’s immediate outcomes were the identification of pathways for selecting and breeding wheat lines with better NO$_3^-$ capturing capacity. This anticipated outcome was achieved.

**Longer term economic outcomes**

Future release of genotypes with improved capacity to capture NO$_3^-$ accumulated in soil over summer and autumn should result in either lower requirements for fertiliser N inputs or better yield in seasons where rainfall moves NO$_3^-$ to depth. Lower input costs or better yields in seasons of high rainfall potentially translate to improved profitability.

**Environmental outcomes**

The annual-based farming systems of southern Australia are characterised by phases of legumes followed by wheat. Typically, soil remains fallow over summer and autumn, periods when large quantities of mineral N (approx.100kg N/ha) can accumulate in soil profiles. Field studies have shown that large quantities of mineral N can move down soil profiles in both sand and sand over clay soils during the early stages of wheat phases, suggesting that the capture of this mineral N is poor. This inefficient use of N added to soil through legumes or applied as fertiliser N causes soil acidification that necessitates costly application of lime. Leaching of NO$_3^-$ in deep drainage leads to contamination of groundwater and subsequent eutrophication of waterways where groundwater outflows occur.

**Achievements/Benefits**

**Background to project**

Wheat is Australia's most important crop. The GRDC is investing substantial resources to support the productivity and sustainability of wheat production, including improved N inputs through better N fixation and more efficient use of fertiliser N. Nitrogen impacts directly on wheat yield and grain quality. Inefficient use of N added to soil through legumes or applied as fertiliser N causes soil acidification. Leaching of NO$_3^-$ in deep drainage leads to contamination of groundwater and contributes to eutrophication of waterways. Since N fertiliser can account for one third of crop production costs, improvements in efficiency of use of N improve profitability as well as environment health.

The annual-based farming systems of southern Australia are characterised by phases of legumes followed by wheat. Typically, soil remains fallow over summer and autumn, periods when large quantities of mineral N (approx.100kg N/ha) can accumulate in soil profiles, sufficient N to support high wheat yields. Field studies have shown that large quantities of mineral N can move down soil profiles in both sand and sand over clay soils during the early stages of wheat phases, suggesting that the capture of this mineral N is poor. This asynchrony in supply and demand for N is a key feature of Australian legume-based farming systems. Rapid exploitation of accumulated mineral N by subsequent crops is one solution to better match supply and demand for N.
Capeweed has been shown to be more efficient at extracting mineral N despite having relatively fewer roots than wheat, either by rapid early proliferation of roots or greater capacity for extraction in lower parts of the root zone. One objective of the project was to compare, against wheat, the capacity of weed species such as capeweed to capture NO\textsubscript{3}. Another objective was to look for differences in the capacity of commercial wheat lines and to examine the capacity of early growth vigour lines produced in the Graingene project to capture NO\textsubscript{3}. The reasons for higher NO\textsubscript{3} capture in species across lines were also to be delineated.

It is generally accepted that NO\textsubscript{3} influx is proton-coupled. Evidence from kinetic studies indicates that roots have at least two distinct NO\textsubscript{3} uptake systems, one a high-affinity system (HATS) and the other a constitutive low-affinity transport system (LATS). There is now good evidence that the NO\textsubscript{3} uptake HATS has two genetically distinct components with different affinities for NO\textsubscript{3} and different patterns of NO\textsubscript{3} regulation. One component is NO\textsubscript{3} inducible (iHATS) and the other is constitutive expression (cHATS). The iHATS transport system is strongly induced in the presence of an external NO\textsubscript{3} supply, while the cHATS transport system is present in roots that have never been treated with NO\textsubscript{3}. LATS operates under higher external NO\textsubscript{3} concentrations (>1mM). It is present in roots that have never been treated with NO\textsubscript{3} and typically exhibits linear kinetics. In higher plants, the NO\textsubscript{3} uptake system is highly regulated and the genes that encode the NO\textsubscript{3} transporter systems are both NO\textsubscript{3} inducible and feedback-repressible at the mRNA level. It is notable that little information exists on NO\textsubscript{3} transporter activity in wheat roots.

Approaches used

Research was conducted in either the field or under controlled conditions. Detailed studies of NO\textsubscript{3} uptake in respect to wheat root distribution were undertaken in either 2m long x 0.24m diameter columns or 1m long glass-fronted rooting boxes. A flowing nutrient film technique (NFT) that feeds a known concentration of NO\textsubscript{3} to root systems was used to examine the dynamics of NO\textsubscript{3} uptake. Use of 15N enriched NO\textsubscript{3} with this system enabled the short term dynamics of NO\textsubscript{3} uptake to be determined, a prerequisite to assessments of root NO\textsubscript{3} transporter activity. These approaches were designed to identify differences in growth rate, specific growth rate, total N uptake, the kinetics of NO\textsubscript{3} uptake, rates of NO\textsubscript{3} reductase and in the architecture of root systems in high and low NO\textsubscript{3} capture wheat lines.

Overview of findings

Initial field work on a deep sand examined plant growth and N uptake of capeweed and wheat lines including Vigour18, an experimental line with early vigour, and commercial wheat varieties, Westonia, Tincurrin, Camm and Janz. Findings from this work have been published in Functional Biology 31: 121-129. An analysis of dynamics of N uptake by capeweed was hampered by poor establishment and only the differences in N uptake by wheat are reported. In summary, shoot biomass of Vigour18 was higher than that of the other genotypes, except for Westonia at booting when 50kg N/ha was applied at three days after wheat emergence. Vigour18 had significantly higher uptake efficiency of fertiliser-N than the other four varieties at tillering when 50kg N/ha was applied. Fertiliser-N uptake efficiency at booting was similar in Vigour18 and Westonia, but significantly higher than expected in three other commercial varieties. Vigour18 had higher root dry matter, root length density and root surface area compared with Janz when examined in columns of soil. The greater root growth of Vigour18 occurred across all soil layers down to 0.6m. Differences in total N uptake between Vigour18 and Janz were apparent from tillering to booting growth stage. Vigour18 also had significantly higher shoot biomass and N uptake compared with Janz when grown in nutrient culture. Nitrate reductase activity (NRA) expressed on a whole plant basis was higher for Vigour18 than for Janz, and was related to total N uptake. However, NRA expressed on per unit fresh weight was not significantly different across the varieties tested. It is concluded that vigorous early root and shoot growth in Vigour18 was the main driving force for higher N uptake.

The success with Vigour18 led to the evaluation of another early vigour line, B18. However, in contrast to Vigour18, B18 did not display improved NO\textsubscript{3} uptake capacity. Two nutrient solution culture experiments, using the NFT system, were conducted to elucidate the kinetics of NO\textsubscript{3} uptake systems in Janz, and early vigour lines Vigour18 and B18. 15N labelling techniques were used to characterise the NO\textsubscript{3} transporter system that was found to be the same in Janz and Vigour18, but lower in B18.

Discussions were held with Canberra-based CSIRO Plant Industry (PI) plant breeders to identify the reasons for the different NO\textsubscript{3} uptake responses of the two early vigour lines. A decision was made to study double haploid crosses of Vigour18 and CM18 (early vigour lines), generated in the Graingene project, since these appeared to give the widest range in growth response to N. Fourteen double haploid crosses were selected on the basis of either low or high tiller capacity, plant height, and leaf width. The objective was to determine whether there were key growth traits and differences in root NO\textsubscript{3} transporter activity that were strongly correlated with NO\textsubscript{3} uptake. The uptake of NO\textsubscript{3} in the double haploid crosses was compared against NO\textsubscript{3} uptake by Kharchia (parent of the early vigour lines), CM18, Vigour18 (early vigour lines) and three commercial

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wheat lines (Wyalkatchem\(^6\), Janz, Mercury). A known concentration of NO\(_3\) (either 0.5 or 1.5mM NO\(_3\) N) was fed using the flowing nutrient film system. Uptake of NO\(_3\) was found to be significantly different across lines. A strong correlation was found between N uptake and plant biomass and leaf area which confirmed earlier findings on the importance of early growth in wheat in NO\(_3\) capture. Other traits including tiller number, specific leaf area, plant height and leaf number were poorly correlated with NO\(_3\) uptake.

The large differences in NO\(_3\) uptake across the double haploid crosses presented an opportunity to examine the range in activity of the low and high affinity NO\(_3\) transporter systems, and to determine whether there was a strong link between transporter activity and N uptake. Six of the double haploid crosses that contrasted in early growth vigour, tillering ability and N uptake, compared to Vigour18, were each fed nine concentrations of 15N labelled NO\(_3\) to facilitate measurements of the activity of low and high affinity NO\(_3\) transporters. Significant differences were detected between genotypes in the rates of uptake of low concentrations of NO\(_3\) in solution (iHATS transporters), whereas there was no difference between varieties and genotypes in the kinetics of NO\(_3\) uptake from solutions containing in excess of 1mM NO\(_3\) N.

The NO\(_3\) capturing capacity of wheat experimental genotypes was examined in the field in 2004 using a deep sand site in the Western Australia (WA) wheat belt (20km SSE of Wongan Hills) that had been instrumented with a weather station and probes to measure changes in soil water content. Sowing was delayed until late June to ensure that early wheat growth occurred during periods of high rainfall that was expected to displace soil NO\(_3\) to depth. Additional NO\(_3\) (30kg N/ha) was applied to ensure that a pulse of NO\(_3\) would be moving within the soil. Soil and plant material was collected 30, 46, 68 and 82 days after sowing (DAS). Wheat genotypes sown included four double haploid crosses from CM18 and Vigour18 (A35, B25, F20, F25), characterised from flowing nutrient film system studies to have either a high to medium (B25, F25) or low iHATS transporter activity (A35, F20). These genotypes were compared against Vigour18, Kharchia and the commercial wheat lines, Wyalkatchem and Janz. Rainfall during June and July 2004 was well below the long term average for the area. As a result, little of the soil-derived NO\(_3\) and applied NO\(_3\) moved to depth during the early growth period of wheat (90kg NO\(_3\) N of the 127kg NO\(_3\) N in the soil to 160cm was in the top 80cm at 30 DAS). Analyses of N uptake by wheat genotypes showed little difference in N uptake between varieties at 30 DAS since this sampling occurred ahead of the major period of N uptake. After 46 DAS, Vigour18 had accumulated 22kg N/ha, whereas Kharchia and Wyalkatchem had taken up only 7-8kg N/ha. Two of the double haploid lines (A35, F25) had taken up 15-17kg N/ha while another two lines (B25, F20) had taken up approx.12kg N/ha that was close to N uptake by Janz. After 46 DAS, the double haploid line A35 had accumulated 48kg N/ha, whereas two other double haploid lines (F20, F25) had N uptake close to that of Vigour18 (approx. 40kg N/ha), while Kharchia and Wyalkatchem contained only 27kg N/ha. After 82 DAS, three of the double haploid lines (A35, B25, F20), Vigour18 and the two commercial lines, Janz and Wyalkatchem, all contained between 50-60kg N/ha.

Two main conclusions can be taken from the work. The kinetics of NO\(_3\) induced transport in roots did not appear to affect the capacity of genotypes to accumulate N in the field. For example, the double haploid line, A35, that possessed low kinetic rates of NO\(_3\) uptake determined in solution culture had the highest NO\(_3\) uptake after 46 DAS. This finding suggests that either superior root growth or higher demand for N induced by higher growth rates were more important determinants of N uptake than root NO\(_3\) transporter activity. Secondly, the capacity of early vigour lines to accumulate N early did not necessarily result in higher N uptake in the longer term, compared to commercial wheat lines, in a season where NO\(_3\) was largely retained within the top 80cm of soil. Further experimentation is required to test the thesis that early growth vigour is advantageous in soils where NO\(_3\) is moved to depth with leaching rainfall.

The role of early accumulation of root biomass in uptake of N was also examined in vigorous and non-vigorous wheat genotypes using glass-walled growth boxes up to the onset of stem elongation. The accumulated total root length down the soil profile was 33% to 83% higher in the vigorous genotypes than in Janz. Although the roots of vigorous and non-vigorous genotypes grew vertically down the soil profile at a similar rate, the roots of vigorous wheat branched earlier and grew horizontally faster and more extensively than those of Janz, resulting in a greater root length density and root number density in the top 0.7m of soil. While the N uptake by roots in the top 0.2m of the soil profile was 60-68% higher in the vigorous genotypes than in Janz, the roots of the vigorous lines located in the layer 0.2-0.7m of the soil profile captured 100% more N fertiliser than those of Janz. No differences in the uptake of N fertiliser by roots located in the layer 0.7-1.0 of the soil profile were obtained. These results indicate that the early and more extensive horizontal growth of the roots in the 0.2-0.7m of the soil profile was responsible for the superior uptake of N by the vigorous lines.

These genotypic differences in root growth and proliferation and their relationship with the early acquisition of N appear to play a significant role in improving the efficiency of N uptake in sandy soils of the Mediterranean climatic region of Australia.
In addition to the work described in the written articles, an additional lysimeter experiment was conducted. This study included two high vigour lines (Vigour18 and B18) in addition to a commercial wheat line (Janz). Plant biomass, leaf area, root length density/root surface area by soil layers, grain yield and NO$_3^-$ by soil layers and NO$_3^-$ trapped in anion resin have been measured. This study confirmed that Vigour18 has superior shoot growth and root length development by depth compared to Janz. However, B18 did not have superior growth characteristics compared to Janz. The discrepancy in performance of the two early vigour lines necessitated studies on the plant traits that contribute to superior uptake of N in early vigour lines. The production of double haploid crosses with a wide array of growth traits as part of the Graingene project provided excellent material for such a study.

Importance of findings to wheat industry
Collectively, the results obtained in this project highlight the importance of adopting screening techniques for vigorous root development as part of selection processes for new wheat genotypes designated for sandplain soils.

Intellectual property summary
The novel double haploid genotypes used in the study are products of the Graingene project. It was agreed that no seed material generated in the project could be released to other parties without the permission of wheat breeders in Graingene.

Additional information

Aust. Farm J. Early root growth key to plant nitrogen uptake, May 2003.