Genotypic variability in wheat for preference of NH_4^+, NO_3^- or $NH_4^+NO_3$

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Abstract
Improving the nitrogen (N) uptake efficiency (NUpE) of wheat has the potential to offer significant economic gains to growers and improvement in environmental quality. One way to improve NUpE may be by matching wheat genotypes which have greater uptake efficiency for NH_4^+ or NO_3^- to soil types that favour the production of one of these N forms over the other. Surprisingly there has been little previous research investigating the genotypic variation in wheat for preference of different forms of N. The aim of this study was to screen a range of wheat cultivars and landraces for their ability to uptake N and produce biomass on NH_4^+, NO_3^- and a mixture of NH_4NO_3. To date, 21 wheat genotypes have been grown hydroponically on three different nutrient solutions providing N as NH_4^+, NO_3^- or NH_4NO_3. The nutrient solutions were all supplemented with a nitrification inhibitor, well aerated and pH controlled. At 8 weeks after sowing (post-tillering for all lines) the shoots were harvested and leaf area, shoot biomass and N content measured. There is significant variation among the genotypes tested in their ability to uptake N and produce biomass on different N sources. Several cultivars, including Frame and Gamenya, produced more biomass with higher N content on NO_3-. Others, including Yitpi and Wyalkatchem performed best on NH_4^+ while others, including Halberd and Condor, performed better on NH_4NO_3 than on either NO_3^- or NH_4^+. This information is critical for further research to determine whether it is possible to improve NUpE by targeting genotypes with differing N preferences to soil types where a particular N form is likely to occur.

Key Words
Nitrogen uptake, ammonium, nitrate, nitrogen preference, root system

Introduction

Given the worldwide interest in improving the nitrogen use efficiency (NUE) of cereals (Hirel et al., 2007; Anbessa and Juskiw 2012), efforts need to be made to improve the nitrogen uptake efficiency (NUpE). Not only is NUpE the primary component of the NUE equation, but N uptake is the process that sets the capital of N through which a cereal crop generates yield and grain protein (Palta and Yang, 2014). Wheat crops in most of the agricultural regions of Australia are inefficient users of the N available in the soil profile at the break of the season, as well as N applied as fertiliser (McDonald 1989; Fillery & McIness 1992; Angus 2001; Sadras & Angus 2006). Studies have shown that wheat genotypes with vigorous early growth have a greater N uptake than current commercial cultivars during the early stages of growth which is associated with an early and fast root growth, root proliferation and root tip production in the top 1m of the soil profile (Liao et al., 2006; Palta and Watt 2009; Palta et al 2011).

Capture of N by root systems is often correlated with root and shoot biomass. However, recent studies by Pang et al (2014) showed that some wheat cultivars, such as Janz, with less root and above ground biomass than vigorous genotypes, had a similar uptake of N. Studies on the kinetics for affinity of NO_3^- (Pang et al 2015) showed that the small root system of Janz had higher affinity for NO_3^- than genotypes with vigorous root systems. Independent studies of wheat in Germany showed that the spring cultivar "Star" not only favoured NH_4^+ as source of N rather than NO_3^-, but its root and shoot growth was greater under NH_4^+ than under NO_3^- (Feil 1994). These findings indicate that the next generation of studies for improving the NUpE in wheat should focus on identifying and exploiting genetic variation for affinity to NO_3^-, NH_4^+ and vigour in the root system.

The overall aim of this study is to improve NUpE by wheat in contrasting soil types, by tailoring genotypes to soils that tend to be dominated by NO_3^- or NH_4^+. We began by identifying and then evaluating, through the use of hydroponics, several wheat genotypes with root systems that have high affinity for NO_3^-, preference for NH_4^+, and/or early vigorous growth.

Methods

Plant material

A random selection of wheat cultivars and landraces, known to have varying NUpE, were chosen for the initial screening. To date 21 genotypes have been tested but this work is ongoing.

Hydroponic testing of plant performance of different N sources

Wheat seedlings were germinated on water agar in the dark for four days. Eight replicate seedlings of each genotype were then transferred to one of three hydroponics systems which were housed a glasshouse with day/night temperatures of 24°C/15°C.

Each hydroponics system was supplied with 100 L of nutrient solution that contained KH_2PO_4 0.2 g/L, $MgSO_4.7H_2O$ 0.4 g/L, K_2SO_4 0.4 g/L, FeEDTA 0.1 g/L, MES 0.5 g/L, $CaCl_2.2H_2O$ 0.4 g/L, $CuSO_4.5H_2O$ 0.00025 g/L, H_3Bo_3 0.001545 g/L, $Na_2MoO_4.2H_20$ 3.63E^{-05} g/L, $ZnSO_4.7H_2O$ 0.001 g/L, $MnSO_4.H_2O$ 0.001 g/L. Each of the three hydroponics systems was supplied with a different N source; KNO3 1.0 g/L or NH4Cl 0.53 g/L or KNO3 0.5 g/L plus NH4Cl 0.27 g/L. The nitrification inhibitor DMPP was added to each solution at 1% of N to prevent ammonia oxidation. The pH of the nutrient solutions were maintained at 6.5 with BlueLab automated pH controllers.

Wheat seedlings were grown for approximately eight week until all genotypes had tillered. Plant N content was measured using a handheld N-Tester at seven weeks. At eight weeks plant were removed from the hydroponics system and their leaf area and shoot dry biomass was measured.

Results

There was genotypic variation in the growth and N uptake among the tested genotypes on the different N forms. Some cultivars, like Frontana and Krichoff, accumulated more shoot biomass and took up more N on NO_3^-. Others, such as Wyalkatchem and Yitpi, performed better on NH_4^+ while Condor, Halberd and Spear, performed better on NH_4NO_3 (Fig 1 and 2).

Figure 3 shows a comparison of shoot dry weight of genotypes grown on NH_4^+ versus NO_3^- which indicates that several genotypes (e.g. Wyalkatchem and Yitpi) grew significantly better on NH_4^+ than NO_3^- while many of the varieties (especially Halberd, Frame and Gamenya) grew significantly better on NO_3^- than NH_4^+ and several genotypes showed no preference (e.g. Mace, Warigal, Janz, Condor). A comparison of leaf N content using this approach gave very similar results (data not shown).

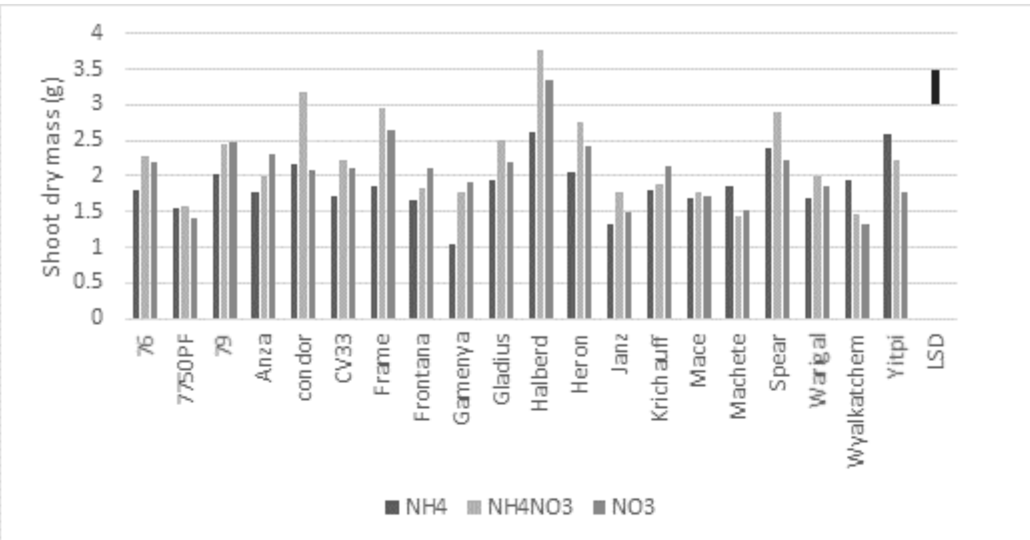

Figure 1. Shoot dry mass production by a variety of wheat genotypes when grown on hydroponics solutions with N supplied as NO_3^-, NH_4^+ or NH_4NO_3. LSD indicates the least significant difference at P= 0.05 from ANOVA (8 replicates for each genotype).

Similar comparisons of shoot weights and leaf N contents of plants grown on NH_4NO_3 versus NO_3^- gave a more linear response (R^2 = 0.62) (data not shown), indicating that most varieties tested show no preference between NH_4NO_3 and NO_3^-. However, a handful of varieties, including Condor and Spear grew significantly better on a mix of NH_4NO_3 than on either NH_4^+ or NO_3^-.

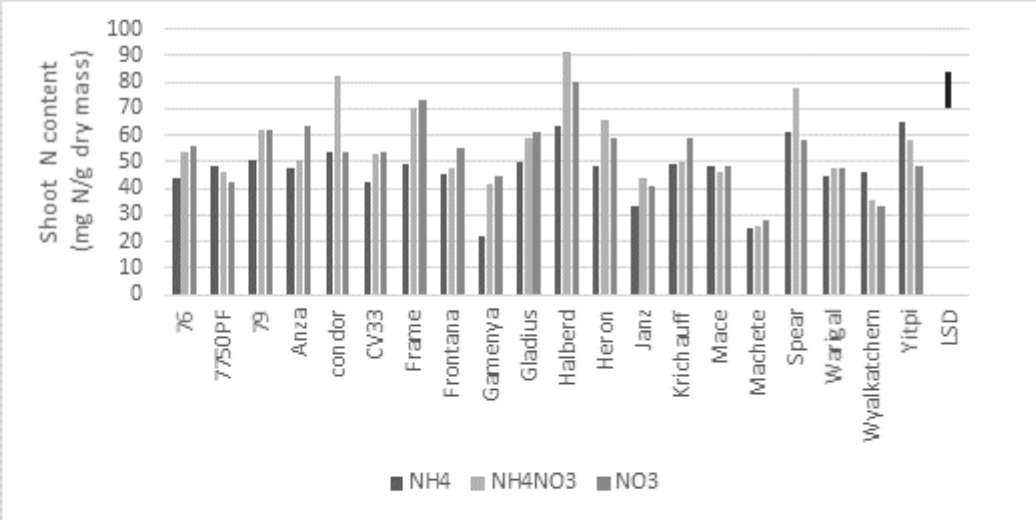

Figure 2. Shoot N contents (as estimated from NTester measurements of leaf greenness) in a variety of wheat genotypes when grown on hydroponics solutions with N supplied as NO_3^-, NH_4^+ or NH_4NO_3. LSD indicates the least significant difference at P= 0.05 from ANOVA (8 replicates for each genotype).

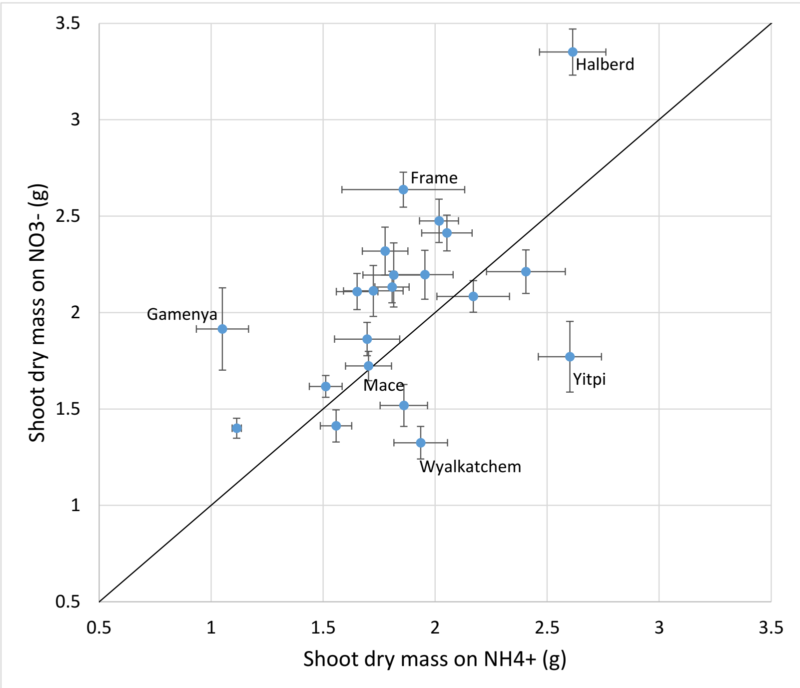

Figure 3. Comparison of shoot dry weight of plants grown on NH_4^+ versus NO_3^-. Genotypes that fall below the 1:1 line produced more biomass on NH_4^+ while those above the line produced more biomass on NO_3^-. Error bars represent the standard error of the mean from 8 replicates. Selected genotypes are indicated with labels.

Next steps

Experiments will be carried out in the near future to confirm these results by tracking the uptake of ^{15}N labelled NH_4^+, NO_3^- or NH_4NO_3 by seedlings of a selection of wheat cultivars. These tests will allow us to assess whether the differences in plant biomass and N content noted in the screening experiments is definitely a result of differences in N uptake when N is present in different redox states.

Conclusions

Surprisingly, there is little information about the genotypic variation for NO_3^- vs NH_4^+ preference among wheat varieties. The data produced in this screening exercise shows that plant biomass accumulation and N content varies among the tested genotypes with some preferring NO_3^-, others NH_4^+ and still others growing better on a mix of the two N forms. This information could be used to develop strategies to improve NUpE by using genotypes with differing N preferences in target soil types where a particular N form is likely to dominate.

Although genotypes with vigorous root systems are able to increase early season N uptake in WA deep sandy soils, they do not improve N uptake in heavy clay soils and in waterlogging-prone Duplex soils (Palta and Yang 2014). It is possible that the profuse root system of these genotypes prefer to take up NO_3^-, but NH_4^+ is likely to dominate in soils prone to waterlogging, soils in which a low pH conditions depress microbial NH_4^+ oxidation and soils that lack organic matter (Schreven and Sieben 1972; Turner et al 2012; Page et al., 2003).

It is expected that genotypes with high affinity for NO_3^- would increase early season N uptake when grown highly aerated sandy soils where nitrification rates are high and the N pool is dominated by NO_3^- . In contrast, in waterlogging-prone Duplex soils, genotypes with high affinity for NH_4^+ are expected to improve N uptake because the low permeability of the B horizons accumulates NH_4^+ rather than NO_3^-. This is important to know because if it turns out to be the case, then pre-breeding and breeding efforts for improving N uptake efficiency and N use efficiency need to be refocussed, paying greater attention to selecting for specific traits for specific environments.

Research of this kind could have impact at a global scale because poor efficiency of N uptake is not only restricted to wheat crops growing in Australian soils, but also in many other wheat growing regions in the world where 30-50% of the N available in the soil profile at the break of the season as well as the N applied as fertiliser is recovered (Raun and Johnson 1999; Dobermann and Cassman 2004).

References

Anbessa Y, Juskiw P (2012) Review: Strategies to increase nitrogen use efficiency of spring barley. *Can. J. Plant Sci*. 92: 617-625Angus JF (2001) Nitrogen supply and demand in Australian agriculture. *Australian Journal of Experimental Agriculture* 41, 277–288.

Dobermann A, Cassman KG (2004) Environmental dimensions of fertilizer N: What can be done to increase nitrogen use efficiency and ensure global food security? p. 261-278. In A.R. Mosier et al. (ed.) Agriculture and the nitrogen cycle: assessing the impacts of fertilizer use on food production and the environment. SCOPE 65. Island Press, Washington,D.C.

Feil B (1994) Growth and ammonium:nitrate uptake ratio of spring wheat cultivars at a homogeneous and a spatially separated supply of ammonium and nitrate. *J. Plant Nutr*. 17, 717-728.

Fillery IRP, McInnes KJ (1992) Components of the fertiliser nitrogen balance for wheat production on duplex soils. *Australian Journal of Experimental Agriculture* 32, 887–899. In "Gaseous loss of nitrogen from plant-soil-systems" (Eds JR Freeney, JR Simpson) pp. 309–325. (Academic Press: San Diego).

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *Journal of Experimental Botany*. 58:2369–2387

Liao, M., Palta, J.A., Fillery, I.R.P., 2006. Root characteristics of vigorous wheat improves early nitrogen uptake. *Aus. J. Agri. Res*.57, 1097-1107.

McDonald GK (1989) The contribution of nitrogen fertiliser to the nitrogen nutrition of rainfed wheat crops in Australia: a review. *Australian Journal of Experimental Agriculture*. 29: 455–481.

Page LK, Dalal RC, Menzies NW (2003) Nitrate ammonification and its relationship to the accumulation of ammonium in vertisol subsoil. Australian Journal of Soil research, 41: 687-697.

Palta JA, Watt M (2009) Crop roots systems form and function: improving the capture of water and nutrients with vigorous root systems. In 'Crop physiology: applications for genetic improvement and agronomy'. (Eds V Sadras, D Calderini) pp. 309–325. (Academic Press: San Diego).

Palta JA, Chen X, Milroy SP, Rebetzke GJ, Dreccer F, Watt M (2011) Large root systems: are they useful in adapting wheat to dry environments? *Functional Plant Biology*. Plant Biol. 38, 347–354.

Palta JA, Yang J (2014) Crop root system behaviour and yield. *Field Crops Research*. 165:1–4

Pang J, Palta JA, Rebetzke GJ, Milroy SP (2014) Wheat genotypes with high early vigour accumulate more N and have higher photosynthetic N use efficiency during early growth. *Functional Plant Biology*. 41, 215-222.

Pang J, Milroy SP, Rebetzke GJ, Palta JA (2015). The influence of shoot and root size on nitrogen uptake in wheat is affected by nitrate affinity in the roots during early growth. *Functional Plant Biology*, 2015, 42, 1179–1189

Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production *Agron. J*. 91 357–63

Sadras VO, Angus JF (2006) Benchmarking water-use efficiency of rainfed wheat in dry environments. *Crop and Pasture Science*. 57: 847-856

Schreven DA, Sieben WH (1972) The effect of storage of soils under water-logged conditions upon subsequent mineralization of nitrogen, nitrification and fixation of ammonia. *Plant and Soil*, 37, 245-253

Turner DA, Edis RE, Chen D, Freney JR, Denmead OT (2012) Ammonia volatilization from nitrogen fertilizers applied to cereals in two cropping areas of southern Australia. Nutrient Cycling in *Agroecosystems* 93(2), 113-126.