Title
Foliar nitrogen application in wheat: the effects on grain N content, recovery of fertilizer and the response of cytosolic glutamine synthetase

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Introduction

New climate and eco-efficient agricultural strategies are under development and include more efficient procedures for nitrogen (N) fertilizer application in the field. These fertilization strategies should minimize the negative environmental impacts of N fertilizers while also providing sufficient N fertilizer for the crop plants to maintain grain yield and protein content.

One such strategy might include the use of foliar application of N fertilizer, in which top-dressings of N are applied to the crop at specific developmental stages, in addition to the solid fertilizer applied to the soil in the beginning of the growing season. This would allow a more refined fertilization strategy which can be tailored to given crops and environmental conditions, taking the actual climatic conditions of the individual growing seasons into account. At the same time, foliar N applications would efficiently boost the protein content of the grains. The major problem associated with foliar application of N fertilizer is the risk of damage (burning) of the leaves and glumes, and resulting yield loss (Gooding and Davies, 1992; Phillips and Mullins, 2004). Accordingly, our research aims to provide a better understanding of the plant physiology and biochemistry resulting from foliar N fertilization which can be used to generate or select genotypes that respond well to foliar application of N fertilizer.

In leaves, inorganic N may be transported into the chloroplast and be assimilated by the chloroplastic glutamine synthase (GS2), or be assimilated directly in the cytosol by the cytosolic GS (GS1). According to many recent observations (Hirel et al. 2001, Yamaya et al. 2002, Martin et al. 2006, Habash et al. 2007), cytosolic GS is one of the key elements controlling N metabolism and plant productivity. In maize, individual GS1 isoforms have been shown to control grain number and grain size (Martin et al. 2006). Wheat GS genes have recently been cloned, characterized, and mapped (Habash et al. 2007, Bernard et al. 2008), but the separate roles of the individual GS isoforms for yield structure and N use in wheat has still not been revealed. It is also not known how the wheat GS genes respond to the application of foliar N fertilizer, and how control of GS expression might be used to reduce leaf burn following foliar N fertilization. These aspects are the subject of our current investigation.

Experimental setup

We have subjected wheat plants to five different N fertilizer regimes. Wheat plants, cv. Amaretto, a registered spring wheat commonly cultivated in Denmark, were grown in 2 L pots (1 plant pot^{-1}) with soil in a growth chamber under 350 µmol m^{-2} s^{-1} photon flux density and 20/17ºC (16/8 h) day/night temperature regime.

The treatments were as outlined in the following and summarised in Table 1. Low N plants (N-) received 240 mg N per plant which was supplied by the soil and was not enriched with $^{15}NH_4{}^{15}NO_3$. High N plants (N+) received an additional 500 mg N (of which 5 mg were ^{15}N), which was added to the soil at flowering. Three treatments using foliar fertilization were carried out in which plants received 170 mg N (of which 1.7 mg were ^{15}N) by foliar application (spraying) of 3 mL 2 M NH_4NO_3 (16%) either at the time of flowering (NF1) or 10 days post flowering (NF2) or at flowering as in NF1 but with the addition of 2 % sucrose to the NH_4NO_3 solution to test the effect of inclusion of carbon (NFC). In addition to the foliar application of N, 330 mg N (without ^{15}N enrichment) were added to the soil at flowering for NF1, NF2 and NFC plants.

Plants were watered twice a week. Prior to sampling of tissues, the plants were washed with tap water on the previous day to remove any remaining N from the surface.

Table 1. Overview of the five treatments used, including the amount of N fertilizer (in mg) available in the soil or subsequently applied to the soil or by foliar fertilization, as well as the time of fertilizer application. The treatments used were low N (N-), high N (N+), foliar application of N at flowering (NF1), foliar application of N and sucrose at flowering (NFC) and foliar application of N at 10 DAF (NF2). DAF, days after flowering.

Treatment	**N available in soil (mg)**	**N applied to soil at flowering (mg)**	**N applied to leaves at flowering (mg)**	**N applied to soil at 10 DAF (mg)**	**N applied to leaves at 10 DAF (mg)**
N-	240				
N+	240	500 (5 as ^{15}N)			
NF1	240	330	170 (1.7 as ^{15}N)		
NFC	240	330	170 (1.7as ^{15}N) + sucrose		
NF2	240			330	170 (1.7 as ^{15}N)

Five replicate plants from each of the five treatments were harvested at the vegetative stage (VS), at flowering (FL), 10 days after flowering (DAF), 20 DAF, 30 DAF and at maturity (M). Each plant was separated into three sections of stem, flag leaves, third leaves, rachis and glumes, including grain. Fresh and dry biomass of the organs of one replicate plant was determined. The tissues of the remaining four replicates were frozen in liquid nitrogen, reduced to a homogenous powder and stored at -80ºC for further use.

Samples from grain and the vegetative part of the plants were analysed using isotopic ratio mass spectrometry coupled with an element analyser (EA-IRMS) to trace ^{15}N. The level of GS1 and GS2 protein in flag leaves was examined by Western blotting using a GS-specific antibody after separation by SDS-PAGE. GS activity measurements were carried out according to the method of O'Neal and Joy (1973).

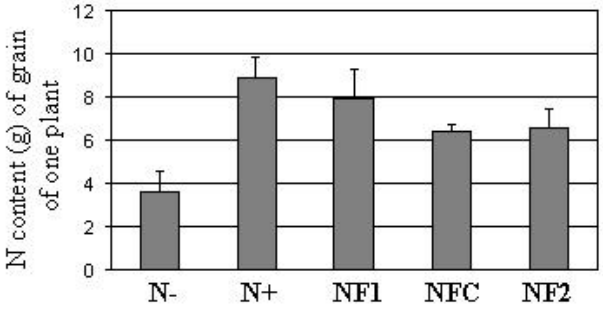
Results and discussion

Leaf burn was observed in plants receiving foliar application of N. Despite the leaf burn, the yield of plants grown under the five different fertilizer conditions was similar in this green house experiment. However, the content of N in the grain at maturity varied with the treatments (Figure 1A) and was slightly lower when a portion of the N fertilizer was applied on the leaves compared to when all N fertilizer was applied to the soil. The foliar application of N fertilizer at 10 DAF resulted in a lower grain N content compared to fertilization at flowering.

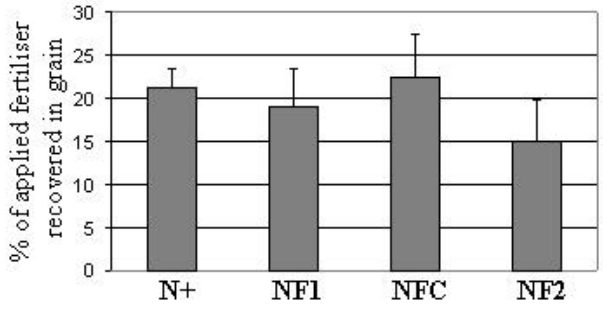
With the use of EA-IRMS and ^{15}N-labelled nitrogen, we observed that about 20% of the applied N fertilizer was recovered in the grain and that the recovery was independent of whether fertilizer was applied to the soil or leaves at flowering (Figure 1B). The application of N fertilizer at 10 DAF, resulted in a lower recovery of N in the grain compared to application at flowering.

Upon analysis of the content of ^{15}N in the vegetative part of the plants at maturity, it became evident that about 50% of the foliar applied N fertilizer remained in the vegetative plant parts at maturity (Figure 1C). This N was absorbed by the plant, but was not translocated to the grain, which suggests a bottleneck in the translocation of N to the grain under these conditions. The distribution of foliar applied N between the vegetative tissues is of current investigation.

A. Grain N content



B. Recovery of fertiliser in the grain at maturity



C. Recovery of applied fertiliser remaining in the vegetative parts at maturity

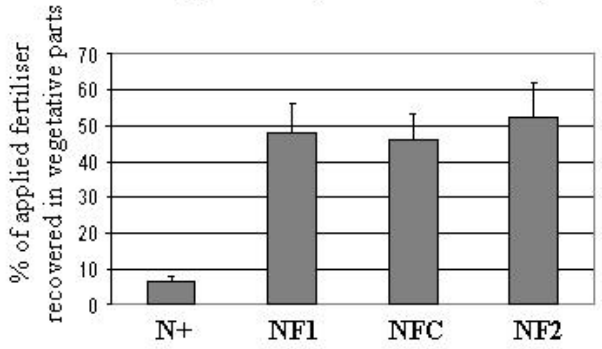


Figure 1. A. Content of N (g) in total amount of grain from one plant at maturity. B. Recovery of applied fertiliser in the grain. The graph shows the percentage of the applied fertiliser that is present in the grain at maturity following translocation. C. Recovery of applied fertiliser in the vegetative plant parts. The graph shows the percentage of the applied fertiliser that remained in the vegetative parts at maturity. Values presented are averages of 4 replicates. Error bars indicate standard error of the mean.

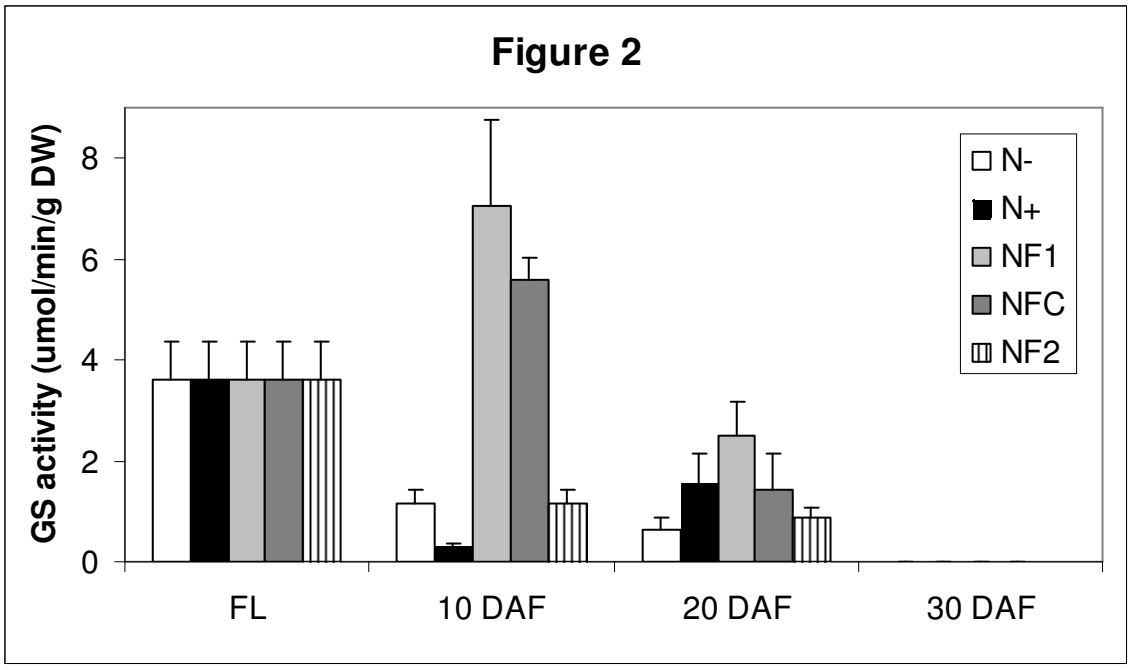


Figure 2: Total GS activity in leaves of wheat plants treated to different N fertilizer regimes measured at the time of flowering and 10, 20 and 30 days after flowering. At 30 DAF, GS activity could not be detected.

The main finding was that GS activity in leaves of plants that had been treated to foliar N fertilization at the time of flowering was nine times as high at 10 DAF compared to plants that were fertilized via the soil (Figure 2). The increase in GS activity occurred despite the fact that GS1 and GS2 protein levels in flag leaf at flowering, 10 and 20 DAF did not differ significantly among the treatments. This suggests a post-translational regulation of GS as previously described (Finnemann and Schjoerring, 2000).

Currently, we are examining the expression of the known wheat GS genes using Q-PCR to examine how gene expression is regulated under the different fertilization regimes. RNA has been extracted from flag leaves at flowering, 10 DAF, 20 DAF and maturity. Gene expression will be analyzed by Q-PCR using primers specifically designed to target GS1 (GS1a, GS1b, GS1c), GSe (GSe1 and GSe2), GSr (GSr1 and GSr2) and GS2 (GS2a, GS2b, GS2c) genes. Further analysis will include quantification of ammonium and nitrate, total amino acid content and amino acid composition.

The results generated in this project will stimulate new research in plant breeding and biotechnology aiming at the development of new wheat genotypes suited for foliar N fertilization that respond efficiently in terms of productivity, nitrogen use efficiency, and grain protein content.

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