# Ammonium nutrition affects the accumulation of winter wheat glutenins

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## 1. Background & Objectives

Bread wheat quality is a highly complex feature which is mainly determined by the amount of grain protein and the qualitative composition of that protein. Nitrogen fertilization is the agronomic practice that most widely affects the quality, since the accumulation of reserve protein is influenced not only by the amount of N fertilizer, but also by the type and timing of N source applied.

Nitrogen fertilization improves grain quality due to a rise in grain protein content (Fuertes-Mendizábal et al., 2011). However, the N source or splitting N application has a more variable effect on grain quality. The main objective of this study was to assess the effect of applying exclusively ammonium as the N source split into two or three applications during the crop lifecycle on the composition of the reserve protein fraction responsible for bread dough strength.

## 2. Materials & Methods

Wheat plants var. Cezanne were sown under greenhouse conditions in 1.5L pots (vermiculite:perlite 1:1). At pre-seeding, 18 mg N were supplied per plant as nitrate ($KNO_3$) or ammonium ($(NH_4)_2SO_4$) to simulate the initial soil N availability under field conditions. P, K and S (188, 188 and 129 mg $plant^{-1}$) were also supplied. Micronutrients and Mg were supplied with the irrigation water (Fetrilon Combi, BASF). The N fertilizer treatments, in Table 1, comprised the same total N application but divided into 2 or 3 applications at different stages along the crop lifecycle according to the Zadoks scale. At harvest, the grain was separated from the straw and milled. Grain N content was determined by combustion with an elemental analyzer (Thermo Finingan). Glutenins were extracted from the flour and separated by RP-HPLC (Figure 1) according to Triboi et al. (2000).

Table 1. N source and rate of the different N-fertilization treatments applied at stages GS20, GS30 and GS37 according to the Zadoks scale.

| Treatment | N source | (mg N $plant^{-1}$) | | |
|---|---|---|---|---|
| | | GS20 | GS30 | GS37 |
| NS | $NO_3^-$ | 11 | 27 | 0 |
| N2S | $NO_3^-$ | 11 | 16 | 11 |
| AS | $NH_4^+$ | 11 | 27 | 0 |
| A2S | $NH_4^+$ | 11 | 16 | 11 |

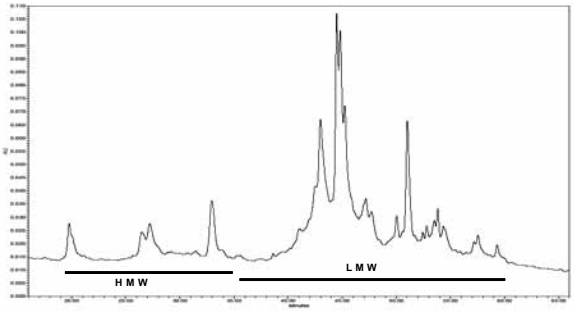


Figure 1. RP-HPLC of var Cezanne glutenins. Peaks eluted after 37 min are LMW-GS, while those eluted before are HMW-GS.

## 3. Results & Discussion

Nitrogen fertilizer applied as ammonium affected positively the grain protein concentration (GP), increasing it by 17.5% compared to the nitrate treatment (Table 2). Thus, despite receiving the same N rate, plants under $NH_4^+$ nutrition produced grains with a higher breadmaking value. Splitting the dose led to an increase in the GP when $NH_4^+$ was applied. So, the third application of $NH_4^+$ at GS37 significantly improved the grain quality compared to those that received only two, applications and

to those that received $NO_3^-$, both in two or three applications. Therefore, the use of $NH_4^+$ as N-source resulted in greater N use efficiency.

Table 2. Nitrogen fertilization management effect on protein and glutenin subunit content. Means in the same column followed by the same letter are not significantly different at P<0.05. ** Interaction N source x splitting at P<0.05.

| | Grain Protein (%) | | Glutenin area | | Glutenin per protein | LMW-GS | HMW-GS | 12 | 9 | 2 | 7* | 12 | 9 | 2 | 7* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | (mV*min per mg flour) | | | | (%) | | | |
| ***N SOURCE*** | | | | | | | | | | | | | | | |
| $NO_3^-$ | 9.12 a | | 270.37 a | | 29.71 a | 231.12 a | 39.26 a | 5.62 a | 7.11 a | 16.32 a | 10.21 a | 14.5 a | 17.7 b | 41.8 b | 26.0 a |
| $NH_4^+$ | 10.72 b | | 309.73 b | | 29.03 a | 268.01 b | 41.72 a | 6.89 b | 6.26 a | 16.11 a | 12.45 b | 16.5 b | 15.0 a | 38.7 a | 29.8 b |
| ***SPLITTING*** | $NO_3^-$ | $NH_4^+$ | $NO_3^-$ | $NH_4^+$ | | | | | | | | | | | |
| **S** | 9.06 a | 10.15 a | 257.4 a | 290.9 a | 28.66 a | 237.12 a | 37.05 a | 5.60 a | 6.17 a | 15.11 a | 10.17 a | 15.2 a | 16.4 a | 41.0 a | 27.4 a |
| **2S** | 9.12 a | 11.23 b | 283.3 a | 328.5 b | 30.08 a | 262.00 b | 43.92 b | 6.90 b | 7.21 a | 17.32 a | 12.49 b | 15.8 a | 16.2 a | 39.5 a | 28.5 a |
| ***NxS*** | ** | | ** | | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Ammonium nutrition increased the glutenin content by 15% (Table 2) but the ratio glutenin/protein was not modified. Splitting the $NH_4^+$ dose also raised glutenin content by 13%, while $NO_3^-$ was applied. The increase in total glutenin content due to $NH_4^+$ nutrition was related to a rise of 16% in the low molecular weight glutenin (LMW-GS) content. The high molecular weight glutenin (HMW-GS) content showed a similar trend, although not significantly so ($p<0.09$ instead of $p<0.05$). Splitting application of $NO_3^-$ or $NH_4^+$ into 3 doses led to an increase in LMW and HMW-GS content by 10% and 18% respectively. Glutenins are, mainly, responsible for the elasticity of the bread dough, so an increment in their content due to $NH_4^+$ nutrition led to an improvement in dough strength and breadmaking quality. The polymorphism of HMW-GS is essentially genetically controlled, but the relationship between the different subunits can be influenced by the environment. In this experiment, the application of $NH_4^+$ changed the quantitative and also the qualitative composition of HMW-GS subunits, favouring the accumulation of subunits 12 and 7*. However, splitting the dose did not change the qualitative composition of the HMW-GS subunits. Therefore, source of N is more important than splitting of the N dose, i.e. the increase in glutenin content due to splitting is not accompanied by a change in glutenin subunit composition.

## 4. Conclusion

The application of an exclusively ammonium N-source, specially when it is split into 3 doses, increases grain glutenin content and produces flours with increased breadmaking strength.

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