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Combined nitrogen and sulphur fertilisation and its effect on wheat quality and protein composition measured by SE-FPLC and proteomics

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Abstract

Soil sulphur deficiency, which is increasingly prevalent in Western Europe, lowers wheat yields, and also affects the gluten quality of the flour. Differences in S availability may change the proportion of S-poor to S-rich gliadins and glutenin subunits. This may cause unpredictable and unwanted variations in wheat quality. The combined effects of nitrogen (N) and sulphur (S) fertilisers and split application of S and N on wheat gluten quality and composition were investigated. The results revealed effects of S fertilisation on dough quality. At high N fertilisation levels significant responses to S fertilisation were found which emphasised the need for precision application of S in intensive wheat production systems. Protein fractionation by SE-FPLC showed that quality differences were associated with changing proportions of high M_r polymeric proteins. Changes in protein composition of salt soluble proteins were also confirmed by proteomics. Glyceraldehyde-3-phosphate dehydrogenase and one of the serpin protein spots increased at high N, combined with the lower S level. The enzymes also increased in samples with increased S fertilisation combined with low N, but was not changed at higher N levels. Furthermore, at high N the serpin protein spot, and also a 27 K protein and one unidentified protein spot decreased with increasing S.

Keywords: Wheat; Sulphur; Nitrogen; Protein composition; Proteomics

1. Introduction

Although wheat has a relatively low sulphur (S) requirement, sub-optimal supply and sometimes also deficiencies have become widespread in Western Europe in recent years. The main reason for this is the decreased inputs of S from industrially generated atmospheric acidic deposition over the last two decades. S deficiency reduces wheat yields and may also affect gluten quality. The proportion of S-poor to S-rich prolamins is dependent on S availability (Castle and Randall, 1987; Fullington et al., 1987; Moss et al., 1981, 1983; Wrigley et al., 1984). Alterations in rheological properties in wheat grown in soils with low S content are recognised even if yield responses of S fertilisations are small or undetectable (Zhao et al., 1999a). Differences in soil S content may therefore cause unpredictable and unwanted variations in wheat quality, which causes difficulties for the milling and baking industry. Optimal S fertilisation management is one important factor in securing good and stable wheat quality.

S assimilation in plants has been described by Zhao et al. (1999a). There is a strong inter-dependence on the plant nitrogen (N) and S metabolism, and plants tend to maintain a relatively constant ratio of organic N to organic S. Proteins constitute about 80% of the organic S, and wheat protein

Abbreviations: Ext, dough extensibility; GS, growth stage; N_{tot}, total N fertilisation (base application + split at any time); R_{max}, dough resistance to extension; S1, S at sowing; S2, S at late application (split application); SDS, SDS sedimentation test; SSDS, specific SDS sedimentation volume; %UPP, percentage unextractable polymeric proteins; Z31, growth stage 31 according to Zadoks decimal code; Z49, growth stage 49 according to Zadoks decimal code; %F1*, peak area of sonicated peak 1 as % of total peak area; %F1, peak area of peak 1 (SDS soluble proteins) as % of total peak area; %F2, peak area of peak 2 as % of total peak area; %F3, peak area of peak 3 as % of total peak area; %F4, peak area of peak 4 as % of total peak area.

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requires about 1 part S for every 15 parts N by weight. Decreased wheat yield responding to sulphur supply has been associated with grain S contents less than 1.2 g S kg^{-1} (Gupta, 1976; Rasmussen et al., 1975), and a N/S ratio greater than 17/1 (Randall et al., 1981). S deficiency induced by N supply can result in accumulation of non-protein N-rich compounds such as amides.

The redistribution of S from vegetative tissues to grain is considerably less than for N. Hocking (1994) found that only about 33% of the S in stem and leaves was re-distributed to the grain, compared to approximately 75% for N. This implies that the S availability needs to be maintained at sufficient levels throughout the whole period of growth to achieve adequate accumulation of S in the grain. Supplying portions of S fertiliser during stem elongation and/or at heading, i.e. split application of S, is a recommended strategy for securing sufficient S in grain and to ensure optimal protein quality.

Bread-making quality is determined by the gluten proteins that form a viscoelastic dough, with a gas retention ability necessary to produce loaves with large volumes and a desired texture. The different types of prolamins comprising the gluten proteins have been classified into the high molecular weight glutenin subunits (HMW-GS), the low molecular weight glutenin subunits (LMW-GS), and the ω-, γ-, and α-gliadins. The HMW-GS and the LMW-GS form the glutenin fraction, consisting of polymeric proteins, whereas the ω-, γ-, and α-gliadins comprise the gliadin fractions and are found as monomers. Differences in bread-making quality of different flours are related to differences in the size distribution of polymeric proteins, and the ratio of monomeric to polymeric proteins. Allelic variation between varieties is one dominating factor causing variation in these attributes (reviewed by Schofield, 1994).

Shewry et al. (1985) classified the prolamins into the S-poor (mainly ω-gliadins), the S-rich (mainly the γ- and α-gliadins and the LMW-GS), and the HMW-GS. Of these, the ω-gliadins are reported to be very low in S-containing amino acids, containing no cysteine or methionine residues (Shewry et al., 1997). The ω-gliadins normally account for 10–20% of the total prolamin fraction. Also the HMW-GS are reported to be low in S, containing 0.5–1.5 mol% of cysteine (Zhao et al., 1999a), mainly located at the ends of the polypeptides. The cysteines play an important role in polymerisation of glutenin subunits to glutenins and to the formation of the gluten network. The HMW-GS account for 6–10% of the total prolamin fraction. The γ- and α-gliadins and the LMW-GS are quantitatively the most abundant, accounting for 70–80% of all prolamins, and contains 2–3 mol% of cysteine. In low S soils a shift in the prolamin composition towards lower proportions of the S-rich and a higher proportions of the S-poor prolamins has been reported (reviewed by Zhao et al., 1999a). Changes in the proportions of single protein types caused by low S availability were recently quantified by Wieser et al. (2004). They found that the amount of ω-gliadins increased considerably, while the amount of HMW-GS increased moderately as S availability was reduced. Furthermore, the amounts of γ-gliadins and LMW-GS were significantly decreased, while only small reductions were found for the α-gliadins. A decreased grain S content has been associated with an increased ratio of HMW-GS/LMW-GS, which in turn shifted the molecular weight distribution of glutenin polymers towards higher M_r (MacRitchie and Gupta, 1993). As the LMW-GS are the major components of the glutenins, the ratio of polymeric to monomeric proteins decreased with lower S content in the grains (MacRitchie and Gupta, 1993; Wieser et al., in press; Zhao et al., 1999b). These compositional changes were associated with rheological changes of the dough, giving increased resistance and decreased extensibility.

As the required dough rheological properties vary according to different products and different processing methods, the effects of grain S content on the final product will be diverse. For bread making, the production of hearth bread or pan bread implies different dough rheology requirements. A higher resistance to extensibility ratio has been positively associated with hearth loaves showing both good shape and a high volume (Færgestad et al., 2000; Uhlen et al., 2004). For production of pan loaves using the Chorleywood Bread Process, Zhao et al. (1997, 1999b) found a positive correlation between the grain S status and loaf volume, and that the grain S status was a better indicator of loaf volume than grain N content. However, there is a need for more information about the impact of grain S status for different products and bread-making processes.

Both high yield and good and stable quality are important features in today's wheat market. To reach these goals increased total N fertilisation and split N application have been the major strategies recommended in intensive wheat production. Split applications of N are commonly used, through reducing the amount of N in the early spring application while giving additional N at stem elongation and/or at early tillering. Late application may secure sufficient N availability during grain-filling, and thereby increase the protein content (Hoel et al., 1999). Few studies have, so far, focused on the use of split applications of S to secure S availability during grain filling and thereby obtain good and stable wheat quality.

The aim of this study was to investigate the effects of combined S and N fertilisation, and effects of split applications of S, on wheat gluten quality and protein composition.

2. Materials and methods

2.1. Chemicals

Urea, thiourea, Tris–HCl, 2-iodoacetamide, glycerol, silver nitrate, sodium thiosulphate, sodium carbonate,