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Changes in the glutathione thiol-disulfide status in wheat grain by foliar sulphur fertilization: consequences for the rheological properties of dough

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Abstract

Storage proteins and glutathione in wheat play an important role in gluten network formation and can be modified by supplementation of nitrogen (N) and sulphur (S) in wheat plants. The glutathione thiol-disulfide status and its relationship to the molecular weight distribution wheat polymeric protein and dough rheological properties have been examined after different foliar S fertilizations (S derived from micronized elemental S and NS, a mixture of N urea and elemental S) applied at the post-anthesis stage. Changes in levels of reduced glutathione (GSH), glutathione disulfide (GSSG), polymeric protein-glutathione mixed disulfide (PPSSG) were analysed by reversed phase high performance liquid chromatography, during grain development using the wheat cultivars, Soissons and Trémie. During the grain desiccation phase, S supplementation (i) increased the GSSG/GSH ratio by 23–25% (ii) induced PPSSG accumulation, and (iii) decreased the formation of SDS-unextractable polymeric protein (UPP) and its molecular mass distribution. However, simultaneous N and S supplementation results in: (i) a decrease in PPSSG formation by 20–30% and (ii) an increase of UPP by 7–18% by enhancing both the branching of the aggregated proteins and their molecular weight. The mixograph parameters show that all forms of endogenous glutathione are linked to dough weakening and are negatively correlated with dough mixing tolerance, dough strength and consistency, while UPP is positively correlated with dough strength and consistency. These findings indicate that S nutrition influences dynamics of the glutathione forms in the grain and results in modification the degree of polymerization of storage protein. Thus both the changes in the form of glutathione and protein polymerization influence the rheological properties of dough.

Keywords: Wheat; Glutathione; Rheological properties; N and S fertilizers; SDS-unextractable glutenin polymer

1. Introduction

Glutathione (γ-glutamyl-cysteinyl-glycine) is a multi-functional plant metabolite. It is a major reservoir of reduced, non-protein sulphur (S) (Noctor et al., 2002). Glutathione has been investigated over many years, especially for its involvement in plant defence mechanisms (Noctor et al., 2002).

In wheat grain, free glutathione occurs in the reduced (GSH) and oxidised (GSSG) forms, as well as in the form of

Abbreviations: DAA, days after anthesis; DTT, dithiotheitol; FDNB, 1-fluoro-2,4-dinitrobenzene; γ-EC, γ-glutamylcysteine; γ-ECS, γ-glutamylcysteine synthetase; GR, glutathione reductase; GSH, free reduced glutathione; GSH-S, glutathione synthetase; GSSG, free oxidized glutathione; HMW-GS, high molecular weight glutenin subunit; HPSEC, high performance size exclusion chomatography; IAA, iodoacetic acid; LMW-GS, low molecular weight glutenin subunits; MALLS, multi angle laser light scattering; MPT, imidline peak time; MPV, midline peak value; MTxI, midline time integral at 12 min after mixing; MTxV, midline time value at 12 min after mixing; MTxW, midline time width at 12 min after mixing; $\langle M \rangle_w$, weight-average molecular weight; N, nitrogen; NS−, foliar N (30 kg N/ha)+S (5 kg S/ha) treatment; NS+, foliar N (30 kg N/ha)+S (10 kg S/ha) treatment; PCA, perchloric acid; PSSG, protein-glutathione mixed disulphides; PPSSG, polymeric protein-glutathione mixed sulphide; $\langle Rg^2 \rangle^{0.5}w$, weight-average mean square radius; RI, refractive index; S, sulphur; S−, foliar S treatment of 5 kg S/ha; S+, foliar S treatment of 10 kg S/ha; SDS, sodium dodecyl sulphate; UPP, unextractable polymeric protein; WS, weakening slope.

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protein-glutathione mixed disulphides (PSSG) (Chen and Schofield, 1995). High PSSG content is observed mainly in the polymeric protein residue when flour is fractionated by a modified Osborne fractionation procedure (Chen and Schofield, 1995; Li et al., 2004; Rhazi et al., 2003).

During grain development, the ratio GSSG/GSH and PSSG formation increases. This effect reduces the occurrence of polymeric protein in the grain, due to their reduced molecular mass distribution and branching of the aggregates (Rhazi et al., 2003). Indeed, free GSSG reacts with the sulphydryl groups of gluten proteins, releasing GSH which may in turn cleave inter-chain disulphide bonds of glutenin polymers (Kranner and Grill, 1996). This can lead to 'depolymerization' of glutenins, which lowers the elasticity of the dough (Coventry et al., 1972) by 'depolymerization' of glutenin polymers. Hüttner and Wieser (2001) confirmed the existence of a SH/SS-interchange in dough induced by GSH. Indeed, it was found that the level of PSSG in gel protein from flours with poor breadmaking performance was consistently higher and significantly different from that of flours with good breadmaking performance (Li et al., 2004). Moreover, improved breadmaking performance due to short-term storage of flour has also been found to be associated with decreased flour GSH and PSSG (Chen and Schofield, 1996).

The effects of S nutrition on dough properties are mainly attributed to its influence on prolamin composition (MacRitchie and Gupta, 1993). Moreover, Koehler and Wieser (2003) have shown that the quantitative distribution of individual gluten protein types is strongly influenced by S fertilization. The increased dough resistance as a result of S deficiency is due to an increase of ratio of high molecular weight glutenin subunit (HMW)/low molecular weight glutenin subunits (LMW) and a consequent shift of polymeric proteins toward higher molecular weight (Koehler and Wieser, 2003; MacRitchie and Gupta, 1993). The decrease in dough extensibility is related to decreases in the proportion of LMW glutenins and some gliadins, such as α-, β-, and γ-gliadins (Shewry and Tatham, 1997). Further, in a previous work (Tea et al., 2003), it was shown using the ^{34}S isotope as a tracer that foliar S fertilizers applied at anthesis stage were incorporated mainly into grain storage proteins, modifying the prolamin composition and their degree of polymerization. In turn these changes influence dough mixing properties.

Apart from affecting prolamin composition, another possible way in which S nutrition may affect dough properties could be by influencing the contribution of S to the forms of glutathione. Indeed, glutathione is a key factor in S metabolism (Noctor et al., 2002) and can be influenced by S supply of wheat. To our knowledge, no studies have demonstrated that S applications also modify the concentration of endogenous glutathione in the flour, thus contributing to modification of dough properties.

The objectives of this study were to determine (i) the influence of S foliar fertilizations applied at the post-anthesis stage, on the content of different forms of glutathione and polymeric protein during grain development, (ii) the relationship between the PSSG levels in polymeric proteins and their molecular mass distribution, and (iii) the involvement of endogenous glutathione on dough properties.

2. Materials and methods

2.1. Plant material and treatment

The two wheat cultivars, Soissons and Trémie, used in this study possessed the Glu-D1 subunits 5 + 10 and 3 + 12, respectively. These two cultivars were chosen for their different technological properties. These cultivars were grown in 2001–2002 at the experimental farm of INRA Plaisir-Grignon, in Northern France (48°51′N, 1°58′E).

A randomized complete block design with three replicates (plots of 35 m^2) and four foliar treatments was used in the experiment. Each replicate had five plots corresponding, respectively, to a control without foliar fertilization (Control), a foliar S treatment with 5 kg S/ha (S−), a foliar S treatment with 10 kg S/ha (S+), a foliar N (30 kg N/ha) + S (5 kg S/ha) treatment (NS−), and a foliar N (30 kg N/ha) + S (10 kg S/ha) treatment (NS+).

At tilling, ear emergence and flag leaf emergence, different soil N fertilizations (NH_4NO_3) were applied at: 60, 120, and 40 kg N/ha, respectively. No S fertilization was applied during this period.

At post-anthesis stage (18 DAA, day after anthesis), foliar fertilizations of S treated plots was applied using elemental S (micronized elemental S mixed with dispersing agents and sodium lignosulphonate) (Microthiol, Cerexagri S.A, Vernon, France). In foliar fertilizations of NS treated plots both urea and elemental S were applied (Nutrithiol Grande Paroisse S.A, Paris, France).

Thirty spikes per replication were collected in each plot, at 8, 22, 36, 48, 59 and 70 DAA. The fresh weight of 30 spikes was recorded before oven drying at 80 °C to a constant weight after which the dry weight was recorded. The difference between these two weights gave the water content, in mg/spike, at each time. For all the biochemical determinations, the fresh grains harvested at 22, 36, 48, 59 and 70 DAA, were freeze-dried and ground (200 μm) in a Janke A10 grinder (Janke & Kunkel GMBH, Stafen, Germany).

2.2. Quantification and measurement of free glutathione in the grain

2.2.1. Extraction of free glutathione (GSH and GSSG)

All extractions were performed at 4 °C and extracts kept on ice. Extractions were conducted as described by Farris and Reed (1987). Flour sample (0.125 g) was suspended in 5% (w/v) ice-cold perchloric acid (PCA; 1.3 ml). Prior to extraction oxygen was removed from the PCA solution by bubbling O_2-free N_2 though the bulk extractant solution, at 4 °C, using a 1 mm i.d. syringe needle at a rate of ∼50 cm^3