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Rising CO_2 concentration altered wheat grain proteome and flour rheological characteristics

Nimesha Fernando [a], Joe Panozzo [b], Michael Tausz [c], Robert Norton [d], Glenn Fitzgerald [b], Alamgir Khan [e], Saman Seneweera [a,f,*]

[a] Department of Agriculture and Food Systems, Melbourne School of Land and Environment, The University of Melbourne, Water Street, Creswick, Victoria 3363, Australia
[b] Department of Primary Industries, Natimuk Road, Private Box 260, Horsham, Victoria 3401, Australia
[c] Department of Forest and Ecosystem Science, Melbourne School of Land and Environment, The University of Melbourne, Water Street, Creswick, Victoria 3363, Australia
[d] International Plant Nutrition Institute, 54 Florence St, Horsham, Victoria 3400, Australia
[e] Australian Proteome Analysis Facility (APAF), Level 4, Building F7B, Research Park Drive, Macquarie University, Sydney, NSW 2109, Australia
[f] Centre for Systems Biology, University of Southern Queensland, Toowoomba, QLD 4350, Australia

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ABSTRACT

Wheat cv. H45 was grown under ambient CO_2 concentration and Free Air CO_2 Enrichment (FACE; e[CO_2], ~550 μmol CO_2 mol^{-1}). The effect of FACE on wheat grain proteome and associated changes in the flour rheological properties was investigated. A comparative proteomic analysis was performed using 2-D-DIGE followed by MALDI/TOF-MS. Total grain protein concentration was decreased by 9% at e[CO_2]. Relative abundance of three high molecular weight glutenin sub units (HMW-GS) were decreased at e[CO_2]. In contrast, relative abundance of serpins Z1C and 1-Cys peroxiredoxin was increased at e[CO_2]. Elevated [CO_2] also decreased the bread volume (by 11%) and dough strength (by 7%) while increased mixing time. However, dough extensibility and dough stability were unchanged at elevated [CO_2]. These findings suggest that e[CO_2] has a major impact on gluten protein concentration which is associated lower bread quality at e[CO_2].

1. Introduction

The current atmospheric carbon dioxide concentration ([CO_2]) has reached the level of 400 μmol CO_2 mol^{-1} and is predicted to be ±550 μmol CO_2 mol^{-1} by the middle of the 21st century according to the Intergovernmental Panel on Climate Change (IPCC) under "mid-range" emission scenario A1B (Carter, Jones, & Lu, 2007). This will have a direct impact on the growth and development and yield formation of crops, particularly for C_3 plants including wheat and rice. It is predicted that grain yield will increase by 15–17% under an atmospheric CO_2 concentration (a[CO_2]) of about 550 μmol CO_2 mol^{-1} (Leakey et al., 2009). However, the positive influence of [CO_2] on plant growth and grain yield is counteracted by inferior grain quality (Fernando, Panozzo, Tausz, Norton, Fitzgerald, Myers, et al., 2012; Fernando, Panozzo, Tausz, Norton, Fitzgerald, & Seneweera, 2012; Högy, Wieser, et al., 2009).

Mostly, wheat is consumed after processing, therefore, end product quality is important (Shewry, 2009). Wheat end product quality is dependent on the functional properties of flour, which is mainly determined by grain protein concentration and composition (Shewry, 2009). Protein concentration in wheat grains varies from 8% to 20% and the major protein fractions can be classified into three main groups, namely structural, metabolic and storage proteins based on their functional characteristics (Shewry, Tatham, Forde, Kreis, & Miflin, 1986). Storage proteins are considered as gluten proteins, which form viscoelastic networks during dough mixing and are highly correlated with the rheological properties of wheat flour (Shewry, 2009).

According to the primary structure, gluten protein can be classified into three main groups, each group consisting of two or three protein types: high molecular weight (HMW) prolamins (consisting of *x*- and *y*- type HMW glutenin subunits), S-poor prolamins (comprises ω-gliadins) and S-rich prolamins (includes α-, β-, and γ- type gliadins and low molecular weight (LMW) glutenin subunits) (Shewry et al., 1986). Gliadins are mainly monomeric proteins that make up 35–45% of the total wheat protein (molecular weight range from 28 to 55 kD) and are soluble in aqueous alcohol. Glutenins are insoluble and are larger polymeric,

* Corresponding author at: Centre for Systems Biology, University of Southern Queensland, Toowoomba, QLD 4350, Australia. Tel.: +61 746315525, mobile: +61 401879853.
E-mail address: saman.seneweera@usq.edu.au (S. Seneweera).

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aggregated proteins linked by disulphide bonds (Shewry & Halford, 2002) mainly associated with dough strength and extensibility (Wieser, Antes, & Seilmeier, 1998). Synthesis of gluten proteins (different fractions) is strongly influenced by genotype, growth conditions and fertilizer application (Wieser, Manderscheid, Erbs, & Weigel, 2008). It has been previously reported that elevated [CO_2] (e[CO_2]) also alters the wheat grain proteome under well watered, high yielding and temperate conditions (Högy, Zorb, Langenkamper, Betsche, & Fangmeier, 2009). However, there are no such data on the impact of rising CO_2 on wheat grain protein quality under low rainfall Mediterranean climate conditions which cover a significant proportion of the global wheat growing areas (Braun, Rajaram, & Ginkel, 1996).

Limited water supply in rain-fed agriculture, common in many wheat growing regions, is predominant in the Mediterranean and semi-arid Australian wheat-belt. It was reported that larger reduction of grain protein concentration under e[CO_2] in semi-arid conditions than under higher rainfall conditions (Fernando et al., 2012).

Furthermore, Mediterranean and semi-arid growing conditions commonly coincide with high temperatures and drought episodes during the crop growing season (Nicolas, Gleadow, & Dalling, 1984), and such events are predicted to increase in the future (Mpelasoka, Hennessy, Jones, & Bates, 2008). Such dry and hot spells, if occurring during grain filling, can have a significant impact on grain protein composition (Panozzo & Eagles, 2000). Yet it has not been evaluated how rising [CO_2] will affect wheat flour protein concentrations, protein quality and the associated changes in rheological characteristics in low rainfall areas under a Mediterranean climate. The current study was conducted in the AGFACE (Australian Grains Free Air Carbon Dioxide Enrichment) facility established in Horsham (Victoria, Australia) within the major wheat production area (Mollah, Norton, & Huzzey, 2009). This area receives only 250–300 mm of rainfall during the growing season, making it one of the driest grain FACE experimental locations in the world. Furthermore, in Mediterranean climate conditions, high temperatures (22–30 °C) often dominate during grain filling. In these experiments, the following hypotheses were tested: (1) e[CO_2] will modify the wheat grain proteome under Mediterranean rain-fed conditions and (2) changes in wheat grain proteome lead to changes in the rheological properties of wheat flour.

2. Materials and methods

2.1. Experimental conditions and CO_2 exposure

The experiment was conducted under field conditions in the AGFACE (Australian Grains Free Air CO_2 Enrichment) facility in Horsham, Victoria, Australia (36°45′07″S, 142°06′52″E; 128 m above sea level) during the 2009 growing season. Four 16 m diameter a[CO_2] plots (“rings”, ±389 μmol CO_2 mol^{-1}) and four e[CO_2] rings (±550 μmol CO_2 mol^{-1}) were used for this experiment. Wheat (*Triticum aestivum* L. cv. H45) was grown on subplots (4 m long × 1.8 m width) in each ring. Carbon dioxide concentration in the FACE rings was maintained at 550 ± 10% μmol CO_2 mol^{-1} by injecting pure CO_2 into the air from an octagonal FACE ring as described by (Mollah et al., 2009). CO_2 enrichment started at crop emergence and continued until physiological grain maturity (DC90, according to the Decimal Code system given by (Zadoks, Chang, & Konzak, 1974). Seeds were sown on 23 June 2009, and crops were harvested on 04 December 2009.

2.2. Climatic and growth conditions during plant growth

The climate type of the region is considered ‘Mediterranean’ with an average annual rainfall at the experiment site of 427 mm (1981–2010), whereby around 250–300 mm rainfall was received in the winter crop growing season, between May and November (Bureau of Metrology 2010). The soil type of the experimental site is Vertosol according to the Australian Soil Classification (Isbell, 1996). Soil consists of ~35% clay at the surface, increasing up to 60% at 1.4 m depth. Total crop duration was 165 days and post anthesis grain filling duration was 38 days. During the growing season, crops received a total of 224 mm of rainfall of which 73 mm was received during the grain filling period (50% flowering to physiological maturity). Daily minimum and maximum air temperatures averaged over the cropping season were 6.2 °C and 19.4 °C respectively. Daily minimum and maximum air temperatures during grain filling period were 11.6 °C and 29.9 °C respectively. Total growing degree days (GDD) during cropping season were 1287 °C and during grain filling period 620 °C. Total GDD were calculated by summing daily degree days according to (Darroch & Baker, 1990). Daily degree days were calculated as $T_n = (T_{max} + T_{min})/2 - T_b$, where T_{max} and T_{min} are the maximum and minimum daily temperatures, respectively, and T_b is the base temperature (5 °C). All agronomic practices in this experiment were similar to normal farming practices of the region. N fertilizer was applied at a rate of 50 kg nitrogen ha^{-1} which was considered to be adequate for prevailing growing conditions.

2.3. Crop harvesting, yield and analysis of protein

Wheat plants, from a 0.5 m^2 ground area were harvested at DC90. Spikes were separated and dried at room temperature. Grains were separated from spikes and aspirated to remove the remaining husk and dust (Vacuum separator, Kimseed, Australia). The total protein concentration in the whole-grain was determined by Near Infrared Reflectance Spectroscopy (NIR, Foss, Sweden) (AACC, 2000) and expressed on a dry weight basis. Grain protein yield (grain protein concentration × grain yield/m^2) (g m^{-2}) was calculated.

2.4. Grain milling and flour mixing properties

Grain samples (30 g) were tempered for 24 h at 13.5% moisture and milled on a Quadrumat Junior Mill (Brabender, OHG Duisburg, Germany). Flour was separated from the bran through a 0.2 mm size sieve. Mixograph analysis was conducted on a 10 g reomixer (Reologica Instruments, Sweden) and mixing properties were obtained. Some of the reomixer parameters are directly related to the basic rheological characteristics of the mixing properties. For example, “peak time” describes the mixing requirements, “peak height” is a measure of dough strength, and “breakdown” reflects dough stability and “peak width” measures dough extensibility (Bohlin, 2007). Reomixer mixing parameters have been shown to be useful for estimating bread volume; using multivariate analysis, bread volume can be predicted to 91% explained variance (Bohlin, 2007). Therefore, calculated bread volume from mixographs was used in the interpretation of the results. Mixograph absorption was expressed on a 12.5% moisture basis.

2.5. Protein extraction

Air-dried wheat grains were ground to fine flour in a commercial blender. Wheat flour (50 mg) samples were dissolved in 700 μl of protein extraction buffer (5 M urea, 2 M thiourea, 2% CHAPS 1% (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate), ASB-14 2% (amidosulfobetaine-14) SB 3–10, 65 mM DTT (dithiothreitol), 40 mM Tris, 10% iso-propanol with protease inhibitor cocktails (Sigma) in a 2 ml screw capped tube containing 0.5 g of acid washed glass beads (Sigma). Beads were beaten for 45 s at 6.5 outputs on Bio 101 (SAVANT) and placed on ice for 10 min and this step was repeated twice. Acrylamide