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Temperature variations during grain filling obtained in growth tunnel experiments and its influence on protein content, polymer build-up and gluten viscoelastic properties in wheat

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ABSTRACT

The aim of this study was to investigate effects of temperature during grain filling on gluten quality characteristics at a lower to moderate temperature range. Experiments with two wheat varieties grown in field covered by polypropylene tunnels during grain filling were performed in two seasons. Mean day temperature differences achieved within the tunnel were approximately 2–2.5 °C from the open to the closed end. There were significant effects of temperature on grain maturity time, thousand grain weight and protein content. The resistance to stretching of the gluten doughs increased with the increasing day temperatures. This was reflected in the proportion of unextractable polymeric proteins (UPP). The results suggest that increases in temperature within this temperature range affect the polymerization of polymeric proteins, giving higher molecular weights, and hence increased Rmax and stronger gluten. The two varieties differed in their response to temperature. In addition, there were seasonal variations in gluten functionality that may be associated with fluctuations in day temperatures between the seasons.

1. Introduction

The viscoelastic property of gluten is crucial for the baking quality of wheat flour. The well-known variation between wheat varieties is utilized to obtain flours of different gluten strengths adapted for different baked products. Not only the variety itself can affect the viscoelasticity of gluten, but also environmental factors linked to the growth conditions during plant development. Several studies have shown large variation between wheat samples of the same variety when grown in different growth environments (Graybosch et al., 1995; Johansson et al., 2002; Moldestad et al., 2011; Uhlen et al., 2004). Such variation is challenging for the industry as it causes an inconsistency in gluten strength that may impede the sorting of the wheat into uniform quality classes. Hence, environmentally induced variation in gluten quality presents a challenge for the milling industry making it difficult to maintain a stable quality, as well as for the bakeries that need to adjust baking processes to compensate for the variations.

The viscoelastic properties of gluten are primarily related to the ratio of monomeric to polymeric proteins (Uthayakumaran et al., 2000) and to the proportion of glutenin aggregates above a certain molecular weight (Southan and MacRitchie, 1999). Particularly, the proportion of the large and unextractable polymeric proteins (UPP) is related to gluten strength of flours. This fraction increases rapidly during the desiccation phase in the developing grain, through post-translational polymerisation of the glutenin subunits (Carceller and Aussenac, 1999). Allelic variation, particularly in the genes encoding the HMW glutenin subunits, is known to affect the degree of polymerisation of the glutenins and thus relate to differences in gluten quality between varieties (Payne, 1987; Shewry et al., 1992). Whereas the varietal differences in gluten viscoelastic properties are well studied and have been linked to specific alleles, the differences caused by environmental factors are less understood. It is suggested that environmental factors may influence both the synthesis of gliadins and glutenin subunits during grain development as well as their polymerisation and formation of the large and insoluble glutenin aggregates (Carceller and Aussenac, 2001; Johansson et al., 2005).

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List of abbreviations

%P	protein concentration in % d.m.
Ext	extensibility as measured by SMS/Kieffer Dough and Gluten Extensibility Rig
FN	Falling Number
Mono/Poly	ratio of monomeric to polymeric proteins
PCA	Principal component analysis
PC	Principal component
Rmax	Resistance to stretching as measured by the SMS/Kieffer Dough and Gluten Extensibility Rig
SE-FPLC	Size Exclusion Fast Performance Liquid Chromatography
SDS	Sodium Dodecyl Sulphate sedimentation volume
TGW	Thousand Grain Weight
TW	Test weight
UPP	Unextractable Polymeric Protein

Temperature during grain filling is among the environmental factors most studied for its impact on the gluten viscoelastic properties. It is well documented that periods of heat stress (>32–35 °C), particularly during the later part of grain filling, reduces the dough strength (Blumenthal et al., 1991). This was found to be due to increased monomer to polymer ratio and/or to increased ratio of HMW to LMW glutenin subunits, leading to lower UPP (Ciaffi et al., 1996). Based on experiments with moderate temperatures in both field and controlled climate chambers, Randall and Moss, 1990 concluded that dough strength generally increases with a temperature up to 30 °C. Other investigations have found results in line with this. For instance, field experiments in Scandinavia have shown that the warmer growth seasons generally give better wheat quality and stronger gluten compared to the cooler seasons (Johansson and Svensson, 1998; Moldestad et al., 2011; Uhlen et al., 2004). However, a positive relationship between growth temperature and gluten quality has not always been supported. Georget et al. (2008) found only limited variation in gluten protein composition from experiments in field and in polythene tunnels mimicking a hot/dry and a wet/cool climate. Several studies have used experiments in controlled climate chambers and analysed the composition and polymerisation state of the gluten proteins. Uhlen et al. (1998) analysed wheat samples from climate chamber experiments with temperature range from 13 to 21 °C, and found increased proportions of polymeric proteins with increasing temperatures, but the differences were moderate. Johansson et al. (2005) did experiments in controlled climate chambers at temperatures of 17/14 °C and 22/19 °C during grain filling, and found no consistent changes in the size distribution of polymeric proteins due to the temperature differences. More recently, Malik and co-workers (Malik et al., 2013, 2011) found increased UPP at the higher temperature regime when analysing grain samples from growth chambers of 22/19 °C and 17/14 °C. In a recent review, Johansson et al. (2013) state that the influence of the temperature on gluten protein structure is complex as it may involve a number of interacting biochemical mechanisms. Varieties may respond differently, but few studies have investigated temperature*genotype interactions when it comes to gluten polymer structure and viscoelastic properties.

The existing literature shows inconsistent results regarding the impact of temperature on gluten quality. Furthermore, few studies have investigated impacts of temperature on gluten polymer structure under field conditions. The approaches taken in the former investigations are primarily experiments in climate chambers with different temperature regimes, or field experiments laid out at different locations and/or in different growing seasons, which are expected to result in different temperature ranges. Both types of experiments may be challenging, the former because the defined conditions in climate chambers may be very different from field conditions, and the latter because a range of abiotic and biotic factors, as well as their interactions, may interfere with the results.

An alternative approach to investigate temperature effects is to use experiments in long growth tunnels, where a temperature gradient is achieved in the longitudinal direction of the tunnel. In such tunnels, plants can be grown in plots in natural soils and be subjected to similar but natural variations in solar radiation and humidity, as well as the seasonal and daily (day/night) variations in temperature. This investigation reports on the effects of temperature variations on gluten quality characteristics that were achieved by using experiments in polypropylene covered tunnels in two different growth seasons. The aims were to investigate the relationships between temperature during grain filling and gluten viscoelastic properties at lower to moderate temperatures, and to explore differences in gluten protein composition and molecular weight distribution of the polymeric proteins.

2. Materials and methods

2.1. Field experiments with polypropylene tunnels during grain filling

Field experiments were performed at Bioforsk Øst, Apelsvoll (60° 42″ N, 10° 51″ E) in Norway in 2010 and 2011. The experimental site is on an imperfectly drained brown earth (Gleyed melanic brunisoil, Canada Soil Survey) with predominantly loam and silty sand textures. Apelsvoll has a mean annual precipitation of 600 mm, a mean annual temperature of 3.6 °C and a mean growing season (May–September) temperature of 12 °C. Two spring wheat varieties, cv. Bjarne (Graminor, Norway) and cv. Zebra (Lantmannen SWseed, Sweden), were used. The field trial covered an area of 6*40 m^2 with a plot size of 1.5*5 m^2. The plots were sown in 4 rows in the longitudinal direction, each containing 8 plots. Sub-blocks of 4 plots having the same variety were randomized in the longitudinal direction of the tunnel, giving a total of 16 plots per variety and two replicates. Thus, each replicate comprised both varieties grown at each of the eight positions in the longitudinal direction of the tunnel. Sowing dates were May 7th (2010) and April 29th (2011). 90 kg nitrogen (N) per ha in a compound NPK fertiliser (Fullgjødsel® 19-4-12, Yara Norge AS) was band-placed in the seedbed at sowing as a basal dressing. In addition, a top dressing of 40 kg N per ha was surface-applied as calcium ammonium nitrate with sulphur (OPTI-NS 27-0-0™, Yara Norge AS) at stem elongation (Zadoks 31). Annual weeds were controlled by spraying once per season, with Ariane S (*fluorxypyr* + *clopyralid* + *MCPA*). In 2010 it was sprayed with fungicides Proline (*prothioconazole*) + Amistar Duo Twin (*azoxystrobin* + *propiconazole*) at the start of flowering. In 2011 the fungicide Stereo (*cyprodinil* + *propiconazole*) was applied together with the insecticide Fastac (*alpha-cypermethrin*) at stem elongation. In addition, it was sprayed at anthesis with fungicide Proline (*prothioconazole*) + insecticide Fastac (*alpha-cypermethrin*). The polypropylene tunnel was established at heading, just prior to anthesis. The tunnel used was a Viking Cathedral tunnel with the size 8 m * 40 m and maximum height of approximately 4 m. The tunnel was closed at the western end, also by polypropylene cloth. Temperature loggers (Temprecord Multitrip™, Multi use temperature recorder) were placed in every second plot (Fig. 1), and positioned at the height of the ears. The experimental area was irrigated at July 13th 2010 with 40 mm, and July 15th 2011 with 25 mm. Irrigation was performed by using a sprinkler system with